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Patentanmeldung Nr. Patent application No. Demande de brevet n°

02023141.1

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

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Anmeldung Nr:
Application no.: 02023141.1
Demande no:

Anmeldetag:
Date of filing: 15.10.02
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

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AUTRICHE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
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Nucleic acids coding for adhesion factors of group B streptococcus, adhesion factors of group B Streptococcus and uses thereof

In Anspruch genommene Prioriät(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

C07K14/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

Nucleic acids coding for adhesion factors of group B streptococcus, adhesion factors of group B streptococcus and uses thereof

The present invention relates to isolated nucleic acid molecules which code for bacterial adhesion factors, the bacterial adhesion factors and various uses thereof.

Background of the Invention

Streptococcus agalactiae, or group B streptococcus (GBS), is a leading cause of infant mortality. GBS encompasses an estimated prevalence of several thousand cases per year resulting in an annual mortality rate in the United States between about 10% and 15% (Schuchat, 1998). Studies from the USA demonstrated a risk of 1-2 cases per 1000 live births (Zangwill *et al.,* 1992) and incidence rates for different European countries vary between 0.24 and 1.26 per 1000 live births (Carstensen *et al.,* 1985; Faxelius *et al.*, 1988). In the United States, up to 30% of pregnant women carry GBS at least temporarily in the vagina or rectum without symptoms (Schuchat, 1998). Infants born to these women become colonized with GBS during delivery (Baker and Edwards, 1995). Aspiration of infected amniotic fluid or vaginal secretions allows GBS to gain access to the lungs. Common manifestations of this infection include bacteraemia, pneumonia, and meningitis (Spellerberg, 2000). Even infant survivors of GBS meningitis suffer from neurologic sequelae ranging from deafness, learning disabilities, as well as motor, sensory, and cognitive impairment (Baker and Edwards, 1995). Currently, antibiotic prophylaxis in parturients is the recommended approach for the prevention of neonatal disease by GBS (Baker *et al.,* 1999); however, with the resurgence of antibiotic resistance in other streptococcal species, a similar plight in GBS may occur.

In addition to infant infections, GBS is also an important pathogen in the elderly and in immunocompromised persons, in which the incidence of invasive GBS disease is about 9 in 100,000 (Farley *et al.*, 1993). Of these infections, the mortality rate can be as high as 30%.

An important GBS virulence determinant is the type-specific capsular polysaccharide, which prevents the deposition of host complement factor C3b and thereby inhibits

opsonophagocytosis of the bacteria (Rubens *et al.*, 1987). Nine distinct capsular serotypes, Ia, Ib, and II to VIII, have been identified so far in GBS (Wessels, 1997). Efforts are currently under way to develop a multivalent conjugate vaccine against GBS based on the capsule polysaccharides of the clinically relevant serotypes (Paoletti *et al.*, 1999; Baker *et al.*, 1999; Baker *et al.*, 2000; Paoletti and Kasper, 2002). However, there are a number of technical difficulties to overcome with capsule-containing conjugate vaccines: multiple serotypes are needed, an appropriate protein conjugate needs to be identified and validated, and potential cross-reaction with human tissues needs to be addressed (Korzeniowska-Kowal *et al.*, 2001). The use of cell surface proteins from GBS represents an attractive alternative to capsule polysaccharides for the development of a vaccine against these bacteria. The surface proteins Sip, Rib, α and β from GBS have already been shown to confer protective immunity in mice against GBS infections (Madoff *et al.*, 1992; Larsson *et al.*, 1997; Larsson *et al.*, 1999; Brodeur *et al.*, 2000). Also two unique surface proteins from a serotype V strain were shown in a mouse model to protect against GBS infection (Areschoug *et al.*, 1999). Finally, antibodies against C5a peptidase from GBS were found to inititate macrophage killing of the bacteria (Cheng *et al.*, 2001).

The interaction of GBS with its host is a complex process involving the colonization and penetration of epithelial and endothelial surfaces and the evasion of the immune defence (Spellerberg, 2000). In streptococci, fibrinogen binding has been shown to play a significant role in the adhesion to host surfaces (Courtney *et al.*, 1994; Cheung *et al.*, 1991; Ni *et al.*, 1998; Pei and Flock, 2001) and the protection from the immune system (Courtney *et al.*, 1997;Thern *et al.*, 1998; Ringdahl *et al.*, 2000). Therefore, several studies have addressed the molecular basis of fibrinogen binding in streptococci of the serological groups A, C and G (Fischetti, 1989; Meehan *et al.*, 1998;Vasi *et al.*, 2000).

Fibrinogen is a 330 kDa glycoprotein found in high concentrations in blood plasma (Fuss *et al.*, 2001; Mosesson *et al.*, 2001). It is a hexamer composed of each of two Aα-, Bβ-, and γ-chains linked together by disulfide bonds. Fibrinogen is a key player in haemostasis and mediates platelet adherence and aggregation at sites of injury. Furthermore, it is cleaved by thrombin to form fibrin, which is the major component of blood clots. Fibrinogen also plays a role in opsonophagocytosis. It has been shown to inhibit the binding of the activated complement factor C3b, thereby blocking the activation of the alternative complement pathway (Whitnack *et al.*, 1984; Whitnack and Beachey, 1985). The newborn's unique

susceptibility for disseminated GBS infections has been associated with a relative complement deficiency (Mills *et al.*, 1979; Edwards *et al.*, 1983). Fibrinogen binding of GBS may thus play an important role in the inhibition of the residual complement activity in the newborn (Noel et al., 1991).

In several studies, the interaction of GBS with human fibrinogen has been demonstrated (Schonbeck *et al.*, 1981; Lammler *et al.*, 1983; Chhatwal *et al.*, 1984; Spellerberg *et al.*, 2002). However, the molecular basis of fibrinogen binding in GBS remained unknown.

GBS has been demonstrated to bind to and invade epithelial and endothelial cells (Gibson *et al.*, 1993; La Penta *et al.*, 1997; Winram *et al.*, 1998). Treatment of GBS with the protease trypsin abolishes the adhesive and invasive properties of the bacteria (Valentin-Weigand and Chhatwal, 1995; Winram *et al.*, 1998), indicating a proteinacious nature of the adhesins and invasins in GBS. As adhesins and invasins are located on the surface of the bacteria and are important for the virulence of GBS, they represent ideal targets for the development of a GBS vaccine.

The problem underlying the present invention was to provide means for the development of medicaments such as vaccines against bacterial infections. More particularly, the problem was to provide new adhesions factors of GBS which can be used for the manufacture of said medicaments.

The problem is solved in a first aspect by an isolated nucleic acid molecule which is selected from the group comprising

a) a nucleic acid having at least 70% identity to a nucleic acid sequence which is selected from the group comprising SEQ ID NO 1 to SEQ ID NO 6.
b) a nucleic acid which is essentially complementary to the nucleic acid of a),
c) a nucleic acid comprising at least 15 sequential bases of the nucleic acid of a),
d) a nucleic acid which anneals under stringent hybridisation conditions to the polynucleotide of a), and
e) a nucleic acid which, but for the degeneracy of the genetic code, would hybridize to the nucleic acid defined in a), b), c) or d).

The problem is solved in a second aspect by an isolated nucleic acid molecule which is selected from the group comprising

a) a nucleic acid having at least 70% identity to a nucleic acid sequence set forth in SeqID NO 7, SeqID NO 8, SeqID NO 9 or SeqID NO 10.
b) a nucleic acid which is complementary to the nucleic acid of a),
c) a nucleic acid comprising at least 15 sequential bases of the nucleic acid of a),
d) a nucleic acid which anneals under stringent hybridisation conditions to the nucleic acid of a), and
e) a nucleic acid which, but for the degeneracy of the genetic code, would hybridize to the nucleic acid defined in a), b), c) or d).

In an embodiment of both aspects of the present invention the identity is at least 80 %, preferably at least 90 %.

In a further embodiment of both aspects of the present invention the nucleic acid is DNA.

In a still further embodiment of both aspects of the present invention the nucleic acid is RNA.

In a preferred embodiment of both aspects of the present invention the nucleic acid molecule is isolated from a bacterium.

In a more preferred embodiment of both aspects of the present invention the bacterium is a species selected from the group comprising *Streptococci, Staphylococci,* and *Lactococci.*

In an even more preferred embodiment of both aspects of the present invention the bacterium is a species which is selected from the group comprising *Streptococcus agalactiae, Streptococcus pyogenes, Streptococcus pneumoniae and Streptococcus mutans*.

In a most preferred embodiment of both aspects of the present invention the bacterium is *Streptococcus agalactiae.*

In an embodiment of the first aspect of the present invention the nucleic acid molecule encodes a fibrinogen-binding-protein comprising at least one repeat of an amino acid motive comprising 16 amino acids.

In an embodiment of the second aspect of the present invention the nucleic acid molecule encodes an adhesion factor which interacts with epithelial cells.

In a preferred embodiment of the first aspect of the present invention the encoded fibrinogen-binding-protein comprises 19 repeats of the amino acid motive whereby the amino acid motive is any one of the ones specified or disclosed herein.

In a more preferred embodiment of the first aspect of the present invention the repeats are encoded by a polynucleotide selected from the group comprising SEQ ID NO21 to SEQ ID No 112.

In a third aspect the problem underlying the present invention is solved by an isolated nucleic acid molecule comprising a nucleic acid sequence, whereby the nucleic acid sequence is selected from the group comprising SEQ ID NO 21 to SEQ ID NO 21 to 112.

In a fourth aspect the problem underlying the present invention is solved by an isolated nucleic acid molecule encoding for a polypeptide whereby the polypeptide comprises an amino acid motive, whereby the amino acid motive is G-N/S/T-V-L-A/E/M/Q-R-R-X-K/R/W-A/D/E/N/Q-A/F/I/L/V/Y-X-X-K/R-X-X (SEQ ID NO 222).

In a fifth aspect the problem underlying the present invention is solved by a vector comprising a nucleic acid according to any aspect of the present invention.

In a preferred embodiment the vector is adapted for recombinant expression of the polypeptide encoded by the nucleic acid.

In a sixth aspect the problem underlying the present invention is solved by a cell comprising the vector according to the present invention..

In a seventh aspect the problem underlying the present invention is solved by a polypeptide comprising an amino acid sequence, whereby the amino acid sequence is encoded by a nucleic acid molecule according to any aspect of the present invention, and fragments of said polypeptide.

In an eighth aspect the problem underlying the present invention is solved by a polypeptide comprising an amino acid sequence, whereby the amino acid sequence is selected from the group comprising SEQ ID NO 11 to SEQ ID NO 20.

In an embodiment of this aspect of the present invention the polypeptide having an amino acid sequence according to any of SEQ ID NO 11 to 16 is a fibrinogen-binding protein.

In a further embodiment of this aspect of the present invention the polypeptide is an adhesion factor which interacts with epithelial cells. In an even more preferred embodiment the epithelial cells are human epithelial cells.

In a ninth aspect the problem underlying the present invention is solved by a polypeptide comprising an amino acid sequence, whereby the amino acid sequence is selected from the group comprising SEQ ID NO 113 to SEQ ID NO 205. In an embodiment the polypeptide comprises at least one of the amino acid sequence according to SEQ ID NO 113 to SEQ ID NO 225 in combination with at least one other amino acid sequence. More preferable this at least one other amino acid sequence is an amino acid sequence according to any of SEQ ID NO 113 to SEQ ID NO 205.

In a tenth aspect the problem underlying the present invention is solved by a polypeptide comprising an amino acid motive, whereby the polypeptide comprises an amino acid motive, whereby the amino acid motive is G-N/S/T-V-L-A/E/M/Q-R-R-X-K/R/W-A/D/E/N/Q-A/F/I/L/V/Y-X-X-K/R-X-X (SEQ ID NO 222).

In an eleventh aspect the problem underlying the present invention is solved by a process for producing a polypeptide according to any aspect of the present invention comprising expressing the nucleic acid molecule according to any of the present invention.

In a twelfth aspect the problem underlying the present invention is solved by a process for producing a cell which expresses a polypeptide according to any aspect of the present invention, comprising transforming or transfecting a host cell with the vector according to the present invention such that the transformed or transfected cell expresses the polypeptide encoded by the polynucleotide contained in the vector.

In a thirteenth aspect the problem underlying the present invention is solved by a pharmaceutical composition, especially a vaccine, comprising a polypeptide or a fragment thereof, as defined in any as pect of the present invention or a nucleic acid molecule according to anyaspect of the present invention.

In a preferred embodiment the pharmaceutical composition comprises an immunostimulatory substance, whereby the immunostimulatory substance is preferably selected from the group comprising polycationic polymers, immunostimulatory deoxynucleotides (ODNs), synthetic KLK peptides, neuroactive compounds, alumn, Freund's complete or incomplete adjuvants or combinations thereof.

In a preferred embodiment the immunostimulatory substance is a combination of either a polycationic anion and immunostimulatory deoxynucleotides or of synthetic KLK peptides and immunostimulatory deoxynucleotides.

In a more preferred embodiment the polycationic polymer is a polycationic peptide and/or whereby the neuroactive compound is human growth hormone.

In a fourteenth aspect the problem underlying the present invention is solved by the use of a polypeptide according to any aspect of the present invention or a fragment thereof for the manufacture of a medicament, especially for the manufacture of a vaccine against bacterial infection.

In a preferred embodiment the bacterial infection is a bacterial infection of *Streptococcus agalactiae.*

In a fifteenth aspect the problem underlying the present invention is solved by the use of molecules which inhibit the binding of a polypeptide according to any aspect of the present invention to fibrinogen for the manufacture of a medicament to prevent and treat bacterial infection. Preferably, the bacterial infection is a *Streptococcus.agalactiae* infection.

In a further embodiment the molecules are selected from the group comprising fibrinogen receptor antibodies, fibrinogen receptor mimotopes and fibrinogen receptor antagonists binding to a polypeptide according to any aspect of the present invention..

In a sixteenth aspect the problem underlying the present invention is solved by the use of molecules which inhibit the binding of a polypeptide according to any aspect of the present invention to epithelial cells, preferably human epithelial cells.

In a seventeenth aspect the problem underlying the present invention is solved by a antibody, or at least an effective part thereof, which binds at least to a selective part of the polypeptide or a fragment thereof according to any aspect of the present invention..

In an embodiment the antibody is a monoclonal antibody.

In a further embodiment said effective part comprises Fab fragments.

In a still further embodiment the antibody is a chimeric antibody.

In a preferred embodiment the antibody is a humanized antibody.

In an eighteenth aspect the problem underlying the present invention is solved by a hybridoma cell line, which produces the antibody according to the present invention .

In a nineteenth aspect the problem underlying the present invention is solved by the use of the antibody according to the present invention for the preparation of a medicament for treating or preventing bacterial infections, especially *Streptococcus agalactiae* infections.

In a twentieth aspect the problem underlying the present invention is solved by an antagonist which reduces or inhibits the activity of the polypeptide or a fragment thereof according to any aspects of the present invention.

In a twenty-first aspect the problem underlying the present invention is solved by a method for identifying an antagonist capable of reducing or inhibiting the activity of the polypeptide or fragment thereof according to any aspect of the present invention comprising:

a) contacting an isolated or immobilized polypeptide according to any of the aspects of the present invention or a fragment thereof. with a candidate antagonist under conditions to permit binding of said candidate antagonist to said polypeptide or fragment thereof, in the presence of a component capable of providing a detectable signal in response to the binding of the candidate antagonist to said polypeptide or fragment thereof; and

b) detecting the presence or absence of a signal generated in response to the binding of the antagonist to the polypeptide or fragment thereof, the presence of a signal indicating a compound capable of inhibiting or reducing the activity of the polypeptide or fragment thereof.

In a twenty-second aspect the problem underlying the present invention is solved by a method for identifying an antagonist capable of reducing or inhibiting the activity of a polypeptide or a fragment thereof according to any the aspects of the present invention comprising:

a) providing the polypeptide according to any aspect of the present invention or a fragment thereof,
b) providing an interaction partner of the polypeptide according to any aspect of the present invention,
c) providing a candidate antagonist,
d) reacting the polypeptide, the interaction partner of the polypeptide and the candidate antagonist, and
e) determining whether the candidate antagonist inhibits or reduces the activity of the polypeptide.

In a twenty-third aspect the problem underlying the present invention is solved by a process for *in vitro* diagnosis of a disease related to expression of the polypeptide or a fragment thereof according to any aspect of the present invention comprising determining the presence of a polynucleotide sequence encoding said polypeptide or the presence of a polypeptide according to any aspect of the present invention.

In a twenty-fourth aspect the problem underlying the present invention is solved by a process for *in vitro* diagnosis of a bacterial infection, preferably *Streptococcus agalactiae* infection, comprising the step of determining the presence of a nucleic acid molecule according to aspect according to the present invention, or of a polypeptide according to any aspect of the present invention.

In a preferred embodiment of the latter two aspects of the present invention the presence is determined in a sample which is preferably derived from a host organism.

In a twenty-fifth aspect the problem underlying the present invention is solved by an affinity device comprising a support material and immobilized to said support material a polypeptide according to any aspect of the present invention or a nucleic acid molecule according to any aspect according to the present invention.

In a twenty-sixth aspect the problem underlying the present invention is solved by the use of a polypeptide according to any aspect of the present invention for the isolation and/or purification and/or identification of an interaction partner of said polypeptide.

In a twenty-seventh aspect the problem underlying the present invention is solved by a of any of the polypeptides according to any aspect of the present invention for the generation of a peptide binding to said polypeptide.

In a preferred embodiment the peptide is selected from the group comprising anticalines.

In a twenty-eighth aspect the problem underlying the present invention is solved by the use of a polypeptide according to any aspect of the present invention for the manufacture of a functional nucleic acid, whereby the functional nucleic acid is selected from the group comprising aptamers and spiegelmers.

In a twenty-ninth aspect the problem underlying the present invention is solved by the use of a nucleic acid molecule according to any aspect of the present invention for the manufacture of a functional ribonucleic acid, whereby the functional ribonucleic acid is selected from the group comprising ribozymes, antisense nucleic acids and siRNA.

As used herein the term SEQ ID NO X to SEQ ID NO Y is an abbreviation for any of the SEQ ID Nos comprised by X any Y including X and Y.

The present inventors have surprisingly found that the genomes of GBS comprises a variety of adhesion factors which share a common amino acid motive. This amino acid motive is responsible for the binding of the adhesion factor to fibrinogen. As used herein, an adhesion factor is a factor, preferable a peptide or a protein which mediates the binding of a microorganism to a substrate. Preferably, the microorganism is GBS. More preferably, the substrate is fibrinogen and a host cell, respectively. The adhesion factor as used herein can be an adhesin or an invasin. The common amino acid motive can be described as follows using the one letter code for amino acids:

G-N/S/T-V-L-A/E/M/Q-R-R-X-K/R/W-A/D/E/N/Q-A/F/I/L/V/Y-X-X-K/R-X-X
(SEQ ID NO 222).

As may be taken from the above sequence the amino acid motive comprises a total of 16 positions. Some of the positions have to be occupied by a distinct amino acid such as, e.g., position 1 or 3 or 4. Other positions such as positions 15 or 16 may be occupied by any amino acid, preferably by a naturally occurring amino acid. These positions are marked in the above sequence with an 'X'. Still further positions can be occupied by different amino acids. These different amino acids are indicated in the above motive, whereby the various amino acids are separated by '/'. Accordingly, at position 2 N, S or T may be present. Any permutations of the above sequence of amino acids can be realized by the one skilled in the art, which are thus within the scope of the present invention.

The present invention is thus related in one aspect to the above amino acid motive. More particularly, the present invention is related to any peptide or polypeptide which comprises this amino acid motive. It is to be understood that the terms peptide and polypeptide are used in a synonymous way if not indicated to the contrary.

Polypeptides, as used herein, include all polypeptides as described below. The basic structure of polypeptides is well known and has been described in innumerable textbooks and other publications in the art. In this context, the term is used herein to refer to any peptide or protein comprising two or more amino acids joined to each other in a linear chain by peptide bonds.

As used herein, unless otherwise indicated, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. It will be appreciated that polypeptides often contain amino acids other than the 20 amino acids commonly referred to as the 20 naturally occurring amino acids, and that many amino acids, including the terminal amino acids, may be modified in a given polypeptide, either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques which are well known to the art. Even the common modifications that occur naturally in polypeptides are too numerous to list exhaustively here, but they are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. Among the known modifications which may be present in polypeptides of the present are, to name an illustrative few, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. Such modifications are well known to those of skill and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as, for instance PROTEINS – STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T.E. Creighton, W.H. Freeman and Company, New York (1993). Many detailed reviews are available on this subject, such as, for example, those provided by Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., *Meth. Enzymol.* 182:626-646 (1990) and Rattan et al., *Protein Synthesis: Posttranslational Modification and Aging*, Ann. N.Y. Acad. Sci. 663:48-62 (1992). It will be appreciated, as is well known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be generally as a result of posttranslational event, including natural processing event and events

brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translation natural process and by entirely synthetic methods, as well. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in *E. coli* or other cells, prior to proteolytic processing, almost invariably will be N-formylmethionine. During post-translational modification of the peptide, a methionine residue at the NH_2-terminus may be deleted. Accordingly, this invention contemplates the use of both the methionine-containing and the methionineless amino terminal variant of the protein of the invention. The modifications that occur in a polypeptide often will be a function of how it is made. For polypeptides made by expressing a cloned gene in a host, for instance, the nature and extent of the modifications in large part will be determined by the host cell posttranslational modification capacity and the modification signals present in the polypeptide amino acid sequence. For instance, as is well known, glycosylation often does not occur in bacterial hosts such as, for example, *E. coli*. Accordingly, when glycosylation is desired, a polypeptide should be expressed in glycosylating host, generally a eukaryotic cell. Insect cell often carry out the same posttranslational glycosylations as mammalian cells and, for this reason, insect cell expression systems have been developed to express efficiently mammalian proteins having native patterns of glycosylation, *inter alia*. Similar considerations apply to other modifications. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. In general, as used herein, the term polypeptide encompasses all such modifications, particularly those that are present in polypeptides synthesized recombinantly be expressing a polynucleotide in a host cell.

Any polypeptide comprising the amino acid motive is regarded as a polypeptide according to the present invention. As explained in greater detail in the example, the present inventors have found that GBS comprises a number of adhesion factors which comprise not only one copy of the amino acid motive but a number thereof. Thus any polypeptide comprising a plurality or being composed of a plurality of the amino acid motive is a polypeptide according to the

present invention. For example, the adhesion factor referred to herein as FbsA may comprise as little as one unit of the amino acid motive to as much as 19 copies thereof.

Other adhesion factors according to the present invention are those referred to herein as PabA, PabB, PabC and PabD. It is to be understood that the term polypeptides according to the present invention also comprise any fragment, derivative or analog thereof. Further preferred polypeptides according to the present invention are those the amino acid sequence of which corresponds to SEQ ID 11 to 20.

The fragment, derivative or analog of the polypeptide of the present invention may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Among the particularly preferred embodiments of the invention in this regard are polypeptides set forth in the Sequence Listing, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments. Additionally, fusion polypeptides comprising such polypeptides, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments, in addition to a heterologous polypeptide, are contemplated by the present invention. Such fusion polypeptides and proteins, as well as polynucleotides encoding them, can readily be made using standard techniques, including standard recombinant techniques for producing and expressing a recombinant polynucleic acid encoding a fusion protein.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are

the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe and Tyr.

Further particularly preferred in this regard are variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragment, having the amino acid sequence of any polypeptide set forth in the Sequence Listing, in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the polypeptide of the present invention. Also especially preferred in this regard are conservative substitutions. Most highly preferred polypeptides having an amino acid sequence set forth in the Sequence Listing without substitutions.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity. Also the polypeptides according to the present invention are preferably isolated polypeptides.

The polypeptides of the present invention include any polypeptide set forth in the Sequence Listing (in particular a mature polypeptide) as well as polypeptides which have at least 70 % identity to a polypeptide set forth in the Sequence Listing, preferably at least 80 % or 85 % identity to a polypeptide set forth in the Sequence Listing, and more preferably at least 90 % similarity (more preferably at least 90 % identity) to a polypeptide set forth in the Sequence Listing and still more preferably at least 95 %, 96 %, 97 %, 98 %, 99 %, or 99.5 % similarity (still more preferably at least 95 %, 96 %, 97%, 98 %, 99 %, or 99. 5 % identity) to a polypeptide set forth in the Sequence Listing and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 5 amino acids and more preferably at least 10, 15 or 16 or multiples thereof. Preferably, the multiples are multiples of a repeat of 16 amino acids, whereby the 16 amino acids correspond to the amino acid motive as disclosed herein.

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides.

Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

Also among preferred embodiments of this aspect of the present invention are polypeptides comprising fragments of the polypeptide having the amino acid sequence set forth in the Sequence Listing, and fragments of variants and derivatives of the polypeptides set forth in the Sequence Listing.

As used herein a fragment is a polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the aforementioned *S. agalactiae* polypeptides and variants or derivatives thereof.

Such fragments may be "free-standing", i. e., not part of or fused to another amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the presently discussed fragments most preferably form a single continuous region. However, several fragments may be comprised within a single larger polypeptide. For instance, certain preferred embodiments relate to a fragment of a polypeptide of the present invention comprised within a precursor polypeptide designed for expression in a host and having heterologous pre and pro-polypeptide regions fused to the amino terminus of the fragment and an additional region fused to the carboxyl terminus of the fragment. Therefore, fragments in one aspect of the meaning intended herein, refers to the portion or portions of a fusion polypeptide or fusion protein derived from a polypeptide or the present invention.

Representative examples of polypeptide fragments of the invention, include, for example, in any selected polypeptide, fragments from about amino acid number 45 – 60, 61 – 76, 77 – 92, 93 – 108, 109 – 124, 125 – 140, 141 – 156, 157 – 172, 173 – 188, 189 – 204, 205 – 220, 221 – 236, 237 – 252, 253 – 268, 269 – 284, 285 – 300, 301 – 316, 317 – 332, 333 – 348, 410 – 414 of the amino acid sequences disclosed herein, or any of the repeats, either alone or in combination with one or several of the ones mentioned in the following tables 1 and 2, optionally combined with the signal peptide or the LPXTG motif.

Table 1:

FbsA of GBS strain 6313	FbsA of GBS strain 706 S2
1 – 35 signal peptide	1 – 35 signal peptide
45 – 60 repeat 1 (SEQ ID 113)	45 – 60 repeat 1 (SEQ ID 132)
61 – 76 repeat 2 (SEQ ID 114)	61 – 76 repeat 2 (SEQ ID 133)
77 – 92 repeat 3 (SEQ ID 115)	77 – 92 repeat 3 (SEQ ID 134)
93 – 108 repeat 4 (SEQ ID 116)	93 – 108 repeat 4 (SEQ ID 135)
109 – 124 repeat 5 (SEQ ID 117)	109 – 124 repeat 5 (SEQ ID 136)
125 – 140 repeat 6 (SEQ ID 118)	125 – 140 repeat 6 (SEQ ID 137)
141 – 156 repeat 7 (SEQ ID 119)	141 – 156 repeat 7 (SEQ ID 138)
157 – 172 repeat 8 (SEQ ID 120)	157 – 172 repeat 8 (SEQ ID 139)
173 – 188 repeat 9 (SEQ ID 121)	173 – 188 repeat 9 (SEQ ID 140)
189 – 204 repeat 10 (SEQ ID 122)	189 – 204 repeat 10 (SEQ ID 141)
205 – 220 repeat 11 (SEQ ID 123)	205 – 220 repeat 11 (SEQ ID 142)
221 – 236 repeat 12 (SEQ ID 124)	221 – 236 repeat 12 (SEQ ID 143)
237 – 252 repeat 13 (SEQ ID 125)	237 – 252 repeat 13 (SEQ ID 144)
253 – 268 repeat 14 (SEQ ID 126)	253 – 268 repeat 14 (SEQ ID 145)
269 – 284 repeat 15 (SEQ ID 127)	269 – 284 repeat 15 (SEQ ID 146)
285 – 300 repeat 16 (SEQ ID 128)	285 – 300 repeat 16 (SEQ ID 147)
301 – 316 repeat 17 (SEQ ID 129)	301 – 316 repeat 17 (SEQ ID 148)
317 – 332 repeat 18 (SEQ ID 130)	378 – 382 LPXTG motif
333 – 348 repeat 19 (SEQ ID 131)	
410 – 414 LPXTG motif	

Table 2:

FbsA of GBS strain 33 H1A	FbsA of GBS strain 176 H4A
1 – 35 signal peptide	1 – 35 signal peptide
45 – 60 repeat 1 (SEQ ID 149)	45 – 60 repeat 1 (SEQ ID 162)
61 – 76 repeat 2 (SEQ ID 150)	61 – 76 repeat 2 (SEQ ID 163)
77 – 92 repeat 3 (SEQ ID 151)	77 – 92 repeat 3 (SEQ ID 164)
93 – 108 repeat 4 (SEQ ID 152)	154 – 158 LPXTG motif
109 – 124 repeat 5 (SEQ ID 153)	
125 – 140 repeat 6 (SEQ ID 154)	
141 – 156 repeat 7 (SEQ ID 155)	
157 – 172 repeat 8 (SEQ ID 156)	
173 – 188 repeat 9 (SEQ ID 157)	
189 – 204 repeat 10 (SEQ ID 158)	
205 – 220 repeat 11 (SEQ ID 159)	
221 – 236 repeat 12 (SEQ ID 160)	
237 – 252 repeat 13 (SEQ ID 161)	
314 - 318 LPXTG motif	

Table 3:

FbsA of GBS strain O90R	FbsA of GBS strain SS1169
1 – 35 signal peptide	1 – 34 signal peptide
45 – 60 repeat 1 (SEQ ID 165)	45 – 60 repeat 1 (SEQ ID 175)
61 – 76 repeat 2 (SEQ ID 166)	61 – 76 repeat 2 (SEQ ID 176)
77 – 92 repeat 3 (SEQ ID 167)	77 – 92 repeat 3 (SEQ ID 177)
93 – 108 repeat 4 (SEQ ID 168)	93 – 108 repeat 4 (SEQ ID 178)
109 – 124 repeat 5 (SEQ ID 169)	109 – 124 repeat 5 (SEQ ID 179)
125 – 140 repeat 6 (SEQ ID 170)	125 – 140 repeat 6 (SEQ ID 180)
141 – 156 repeat 7 (SEQ ID 171)	141 – 156 repeat 7 (SEQ ID 181)
157 – 172 repeat 8 (SEQ ID 172)	157 – 172 repeat 8 (SEQ ID 182)
173 – 188 repeat 9 (SEQ ID 173)	173 – 188 repeat 9 (SEQ ID 183)
189 – 204 repeat 10 (SEQ ID 174)	189 – 204 repeat 10 (SEQ ID 184)
267 – 270 LPXTG motif	205 – 220 repeat 11 (SEQ ID 185)
	221 – 236 repeat 12 (SEQ ID 186)
	237 – 252 repeat 13 (SEQ ID 187)
	253 – 268 repeat 14 (SEQ ID 188)
	269 – 284 repeat 15 (SEQ ID 189)
	285 – 300 repeat 16 (SEQ ID 190)
	301 – 316 repeat 17 (SEQ ID 191)
	317 – 332 repeat 18 (SEQ ID 192)
	333 – 348 repeat 19 (SEQ ID 193)
	349 – 364 repeat 20 (SEQ ID 194)
	365 – 380 repeat 21 (SEQ ID 195)
	381 – 396 repeat 22 (SEQ ID 196)
	397 – 412 repeat 23 (SEQ ID 197)
	413 – 428 repeat 24 (SEQ ID 198)
	429 – 444 repeat 25 (SEQ ID 199)
	445 – 460 repeat 26 (SEQ ID 200)
	461 – 476 repeat 27 (SEQ ID 201)
	477 – 492 repeat 28 (SEQ ID 202)
	493 – 508 repeat 29 (SEQ ID 203)
	509 – 524 repeat 30 (SEQ ID 204)
	586 – 590 LPXTG motif

As used herein "about" includes the particularly recited ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments of the invention include, for example, truncation polypeptides including polypeptides having an amino acid sequence set forth in the Sequence Listing, or of variants or derivatives thereof, except for deletion of a continuous series of residues (that is, a continuous region, part or portion) that includes the amino terminus, or a continuous series of

residues that includes the carboxyl terminus or, as in double truncation mutants, deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Fragments having the size ranges set out above also are preferred embodiments of truncation fragments, which are especially preferred among fragments generally. Degradation forms of the polypeptides of the invention in a host cell are also preferred.

Also preferred in this aspect of the invention are fragments characterized by structural or functional attributes of the polypeptide of the present invention. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions of the polypeptide of the present invention, and combinations of such fragments.

Preferred regions are those that mediate activities of the polypeptide of the present invention. Most highly preferred in this regard are fragments that have a chemical, biological or other activity of the polypeptide of the present invention, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Particularly preferred are fragments comprising a receptor activity for such as, e.g., fibrinogen in case of FbsA or the host cell in case of PabA, PabB, PabC und PabD that confer a function essential for the ability of *S. agalactiae* to cause disease in humans and/or that are able to mediate the adherence of *S. agalactiae* to epithelial cells, more preferably human epithelial cells. Further preferred polypeptide fragments are those that comprise or contain antigenic or immunogenic determinants in an animal, especially in a human. A host cell as used herein is a cell which is capable of uptaking of GBS in the natural host or in an internalization assay such as, e.g., the one as described in example 1.

The polypeptides according to the present invention may be used for the detection of the organisms or organisms in a sample containing these polypeptides. Preferably such detection is for diagnosis, more preferable for the diagnosis of a disease, most preferably for the diagnosis of a diseases related or linked to the presence or abundance of Gram-positive bacteria, especially bacteria selected from the group comprising streptococci, staphylococci

and lactococci. More preferably, the microorganisms are selected from the group comprising *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Streptococcus mutans*.

The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of the polypeptide of the present invention in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of the polypeptide compared to normal control tissue samples may be used to detect the presence of an infection, for example, and to identify the infecting organism. Assay techniques that can be used to determine levels of a polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Among these, ELISAs frequently are preferred. An ELISA assay initially comprises preparing an antibody specific to the polypeptide, preferably a monoclonal antibody. In addition, a reporter antibody generally is prepared which binds to the monoclonal antibody. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, such as horseradish peroxidase enzyme.

The polypeptides according to the present invention may also be used for the purpose of or in connection with an array. More particularly, at least one of the polypeptides according to the present invention may be immobilized on a support. Said support typically comprises a variety of polypeptides whereby the variety may be created by using one ore several of the polypeptides according to the present invention and/or polypeptides being different therefrom. The characterizing feature of such array as well as of any array in general is the fact that at a distinct or predefined region or position on said support or a surface thereof, a distinct polypeptide is immobilized. Because of this any activity at a distinct position or region of an array can be correlated with a specific polypeptide. The number of different polypeptides immobilized on a support may range from as little as 10 to several 1000 different polypeptides. The density of polypeptides per cm^2 is in a preferred embodiment as little as 10 oligonucleotides per cm^2 to at least 400 different polynucleotides per cm^2 and more particularly at least 1000 different polypeptides per cm^2.

The manufacture of such arrays is known to the one skilled in the art and, for example, described in US patent 5,744,309. The array preferably comprises a planar, porous or non-

porous solid support having at least a first surface. The polypeptides as disclosed herein, are immobilized on said surface. Preferred support materials are, among others, glass or cellulose. It is also within the present invention that the array is used for any of the diagnostic applications described herein. Apart from the polypeptides according to the present invention also the nucleic acid molecules according to the present invention may be used for the generation of an array as described above. This applies as well to an array made of antibodies, preferably monoclonal antibodies as, among others, described herein.

The isolated nucleic acid molecule according to the present invention, also referred to herein as the nucleic acid (molecule) according to the present invention, codes for the amino acid motive and the polypeptides according to the present invention. The nucleic acid molecule according to the present invention can in a first alternative be a nucleic acid (molecule) which has an identity of at least 70 % to a nucleic acid molecule which has the nucleic acid sequence as specified in SEQ ID No.1 to 10. It is also within the present invention that the isolated nucleic acid molecule has a similarity of at least 70 % of any sequence which codes for any of the polypeptides of the present invention. Preferably, the identity is at least 80 % and more preferably the identity is at least 90 %. Identity may also be 95%, 96 %, 97 %, 98 %, 99% or 99.5 %.

Identity, as known in the art and used herein, is the relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the mach between strings of such sequences. Identity can be readily calculated (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or two polypeptide sequences, the term is well known to skilled artisans (*Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. *Applied Math*., 48: 1073 (1988)).

Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are codified in computer programs. Preferred computer program methods to determine identity between two sequences include, but are not limited to, GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol.* 215: 403 (1990)).

The nucleic acid according to the present invention can as a second alternative also be a nucleic acid which is at least essentially complementary to the nucleic acid described as the first alternative above. As used herein complementary means that a nucleic acid strand is base pairing via Watson-Crick base pairing with a second nucleic acid strand. Essentially complementary as used herein means that the base pairing is not occurring for all of the bases of the respective strands but leaves a certain number or percentage of the bases unpaired or wrongly paired. The percentage of correctly pairing bases is preferably at least 70 %, more preferably 80 %, even more preferably 90 % and most preferably any percentage higher than 90 %. It is to be noted that a percentage of 70 % matching bases is considered as homology and the hybridization having this extent of matching base pairs is considered as stringent. Hybridization conditions for this kind of stringent hybridization may be taken from Current Protocols in Molecular Biology, John Wiley and Sons, Inc., 1987. More particularly, the hybridization conditions can be as follows:

- Hybridization performed e.g. in 5 x SSPE, 5 x Denhardt's reagent, 0.1% SDS, 100 g/mL sheared DNA at 68°C
- Moderate stringency wash in 0.2xSSC, O.1% SDS at 42°C
- High stringency wash in 0.1xSSC, 0.1% SDS at 68°C

Genomic DNA with a GC content of 50% has an approximate T_M of 96°C. For 1% mismatch, the T_M is reduced by approximately 1°C.

In addition, any of the further hybridization conditions described herein are in principle applicable as well.

The nucleic acid according to the present invention can as a third alternative also be a nucleic acid which comprises a stretch of at least 15 bases of the nucleic acid according to the first

and second alternative of the nucleic acid molecule according to the present invention as outlined above. Preferably, the bases form a contiguous stretch of bases. However, it is also within the present invention that the stretch consists of two or more moieties which are separated by a number of bases.

The nucleic acid according to the present invention can as a fourth alternative also be a nucleic acid which anneals under stringent hybridisation conditions to any of the nucleic acids of the present invention according to the above outlined first, second, and third alternative. Stringent hybridisation conditions are typically those described herein.

Finally, the nucleic acid according to the present invention can as a fifth alternative also be a nucleic acid which, but for the degeneracy of the genetic code, would hybridise to any of the nucleic acids according to any of the nucleic acids of the present invention according to the first, second, third, and fourth alternative as outlined above. This kind of nucleic acid refers to the fact that preferably the nucleic acids according to the present invention code for the polypeptides according to the present invention and thus for adhesins and invasions, respectively. This kind of nucleic acid is particularly useful in the detection and thus diagnosis of the nucleic acid molecules according to the present invention and thus of the respective microorganisms such as GBS and any disease or diseased condition where this kind of microorganims is involved. Preferably, the hybridisation would occur or be preformed under stringent conditions as described in connection with the fourth alternative described above.

Polynulceotide(s) as used herein generally refer to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as used herein refers to, among other, single-and double-stranded DNA, DNA that is a mixture of single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded, or a mixture of single- and double-stranded regions. In addition, polynucleotide as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term polynucleotide includes DNAs or RNAs as

described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, *inter alia*. The term polynucleotide also embraces short polynucleotides often referred to as oligonucleotide(s). "Polynucleotide" and "nucleic acid" or "nucleic acid molecule" are often used interchangeably herein.

Using the information provided herein and known, standard methods, such as those for cloning and sequencing and those for synthesizing polynucleotides and polypeptides (see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989)), one can generate numerous unique fragments, both longer and shorter than the polynucleotides and polypeptides set forth in the Sequence Listing, of the *S. agalactiae* genome and the *S. agalactiae* coding regions, which are encompassed by the present invention. To be unique, a fragment must be of sufficient size to distinguish it from other known nucleic acid sequences, most readily determined by comparing any selected *S. agalactiae* fragment to the nucleotide sequences in computer databases such as GenBank. Such comparative searches are standard in the art. Many unique fragments will be *S. agalactiae* – specific. Typically, a unique fragment useful as a primer or probe will be at least about 20 to 25 nucleotides in length, depending upon the specific nucleotide content of the sequence. Additionally, fragments can be, for example, at least about 30, 40, 50, 60, 75, 80, 90, 100, 150, 200, 250, 300, 400, 500 or more nucleotides in length. The nucleic acid fragment can be single, double or triple stranded, depending upon the purpose for which it is intended.

Additionally, as discussed above and below, modifications can be made to the *S. agalactiae* polynucleotides and polypeptides that are encompassed by the present invention. For example, nucleotide substitutions can be made which do not affect the polypeptide encoded by the nucleic acid, and thus any polynucleotide which encodes the polypeptides of this

invention is within the present invention. Additionally, certain amino acid substitutions (and corresponding nucleotide substitutions to encode them) can be made which are known in the art to be neutral (Robinson W.E. Jr. and Mitchell, W.m., *AIDS* 4: S141-S162 (1990)). Such variations may arise naturally as allelic variations (e. g. due to genetic polymorphism) or may be produced by human intervention (e. g. by mutagenesis of cloned DNA sequences), such as induced point, deletion, insertion and substitution mutations. Minor changes in amino acid sequences are generally preferred, such as conservative amino acid replacements, small internal deletions or insertions, and additions or deletions at the ends of the molecules. Substitutions may be designed based on, for example, the model of Dayhoff, et al. (in *Atlas of Protein Sequence and Structure 1978*, Nat'l Biomed. Res. Found., Washington D.C.). These modifications can result in changes in the amino acid sequence, provide silent mutations, modify a restriction site, or provide other specific mutations. Likewise, such amino acid changes result in a different nucleic acid encoding the polypeptides and proteins. Thus, alternative polynucleotides, which are within the parameters of the present invention, are contemplated by such modifications.

Furthermore, some of the polynucleotide sequences set forth in the Sequence Listing are open reading frames (ORFs), i. e. coding regions of *S. agalactiae*. The polypeptide encoded by each open reading frame can be deduced, and the molecular weight of the polypeptide thus calculated using amino acid residue molecular weight values well known in the art. Any selected coding region can be functionally linked, using standard techniques such as standard subcloning techniques, to any desired regulatory sequence, whether a *S. agalactiae* regulatory sequence or a heterologous regulatory sequence, or to a heterologous coding sequence to create a fusion protein, as further described herein.

Polynucleotides of the present invention may be in the form of RNA, such as mRNA or cRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. The DNA may be triple-stranded, double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The coding sequence which encodes a *S. agalactiae* polypeptide of this invention may be identical to the coding sequence of a polynucleotide set forth in the sequence listing. It also

may be a polynucleotide with a different sequence which, as a result of the redundancy (degeneracy) of the genetic code, encodes a *S. agalactiae* polypeptide set forth in the sequence listing.

Polynucleotides of the present invention which encode a *S. agalactiae* polypeptide as disclosed herein, including those set forth in the sequence listing may include, but are not limited to, the coding sequence for a mature polypeptide, by itself; the coding sequence for a mature polypeptide and additional coding sequences, such as those encoding a leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of a mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to non-coding 5′ and 3′ sequences, such as the transcribed, non-translated sequences that play a role in transcription (including termination signals, for example), ribosome binding, mRNA stability elements, and additional coding sequence which encode additional amino acids, such as those which provide additional functionalities. Thus, for instance, a polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in the pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci., USA 86*: 821-824 (1989), for instance, hexa-histidine provides for convenient purificaion of the fusion protein. The HA tag may also be used to create fusion proteins and corresponds to an epitope derived of influenza hermagglutinin protein, which has been described by Wilson et al., *Cell 37*:767 (1984), for instance. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated genetic elements.

In accordance with the foregoing, the term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides which include a sequence encoding a polypeptide of the present invention, particularly a polypeptide having a *S. agalactiae* amino acid sequence set forth in the Sequence Listing. The term encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by integrated phage or insertion sequence or editing) together with additional regions, that also may contain coding and/or non-coding sequences.

The present invention further relates to variants of the herein above described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having a deducted *S. agalactiae* amino acid sequence set forth in the Sequence Listing. A variant of the polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis techniques, including those applied to polynucleotides, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned polynucleotides by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or on-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Preferred are polynucleotides encoding a variant, analog, derivative or fragment, or a variant, analogue or derivative of a fragment, which have a *S. agalactiae* sequence as set forth in the Sequence Listing, in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid(s) is substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the *S. agalactiae* polypeptides set forth in the Sequence Listing. Also especially preferred in this regard are conservative substitutions.

Further preferred embodiments of the invention are polynucleotides that are at least 70 % identical over their entire length to a polynucleotide encoding a polypeptide according to the present invention and more particularly those polypeptides having an amino acid sequence set forth in the Sequence Listing, and polynucleotides which are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80 % or at least 85 % identical over their entire length to a polynucleotide encoding a *S. agalactiae* polypeptide according to the present invention and more particularly those polypeptides set forth in the Sequence Listing, including complementary polynucleotides. In this regard, polynucleotides at least 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, or 96 % identical over their entire length to the same are particularly preferred, and among these particularly preferred polypeptides, those with at least 95 % are especially preferred. Furthermore, those with at least 97 % are highly preferred among those

with at least 95 %, and among these, those with at least 98 % and at least 99 % are particularly highly preferred, with at least 99 % or 99.5 % being the more preferred.

Preferred embodiments in this respect, moreover, are polynucleotides which encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the DNA set forth in the Sequence Listing.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. Stringent conditions are typically selective conditions. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95 % and preferably at least 97 % identity between the sequences. For a specific sequence, stringent conditions can be determined empirically according to the nucleotide content, as is known in the art and also exemplified herein. For example, a typical example of stringent conditions is hybridization of a 48mer having 55 % GC content at 42°C in 50 % formamide and 750 mM NaCl followed by washing at 55°C in 15 mM NaCl and 0.1 % SDS.

As discussed additionally herein regarding polynucleotide assays of the invention, for instance, polynucleotides of the invention as discussed above, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding polypeptides of the present invention and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the polynucleotides of the present invention. Such probes generally will comprise at least 15 bases. Preferably, such probes will have at least 20, at least 25 or at least 30 bases, and may have at least 50 bases. Particularly preferred probes will have at least 30 bases, and will have 50 bases or less, such as 30, 35, 40, 45, or 50 bases.

For example, the coding region of the polynucleotide of the present invention may be isolated by screening using the known DNA sequence to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the present invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine to which members of the library the probe hybridizes.

The polynucleotides and polypeptides of the present invention may be employed as reagents and materials for development of treatments of and diagnostics for disease, particularly human disease, as further discussed herein relating to polynucleotide assays, *inter alia*.

The polynucleotides of the present invention that are oligonucleotides can be used in the processes herein as described, but preferably for PCR, to determine whether or not the *S. agalactiae* genes identified herein in whole or in part are present and/or transcribed in infected tissue such as blood. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained. For this and other purposes the arrays comprising at least one of the nucleic acids according to the present invention as described herein, may be used.

The polynucleotides may encode a polypeptide which is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

The present invention additionally contemplates polynucleotides functionally encoding fusion polypeptides wherein the fusion polypeptide comprises a fragment of a *S. agalactiae* polypeptide and one or more polypeptide(s) derived from another *S. agalactiae* polypeptide or from another organism or a synthetic polyamino acid sequence. Such polynucleotides may or may not encode amino acid sequences to facilitate cleavage of the *S. agalactiae* polypeptide from the other polypeptide(s) under appropriate conditions.

In sum, a polynucleotide of the present invention may preferably encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor

of a mature protein having one or more prosequences which are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

Isolated as used herein means separated "by the hand of man" from its natural state; i.e., that, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally occurring polynucleotide or a polypeptide naturally present in a living organism in its natural state is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. As part of or following isolation, such polynucleotides can be joined to other polynucleotides, such as DNAs, for mutagenesis, to form fusion proteins, and for propagation or expression in a host, for instance. The isolated polynucleotides, alone or joined to other polynucleotides such as vectors, can be introduced into host cells, in culture or in whole organisms. Introduced into host cells in culture or in whole organisms, such DNAs still would be isolated, as the term is used herein, because they would not be in their naturally occurring form or environment. Similarly, the polynucleotides and polypeptides may occur in a composition, such as a media formulations, solutions for introduction of polynucleotides or polypeptides, for example, into cells, compositions or solutions for chemical or enzymatic reactions, for instance, which are not naturally occurring compositions, and, therein remain isolated polynucleotides or polypeptides within the meaning of that term as it is employed herein.

The nucleic acids according to the present invention may be chemically synthesized. Alternatively, the nucleic acids can be isolated from various microorganisms by methods known to the one skilled in the art. Appropriate sources are, e.g. *Streptococcus agalactiae, Streptococcus pyogenes, Streptococcus mutans* and *Streptococcus pneumoniae*.

The nucleic acids according to the present invention may be used for the detection of nucleic acids and organisms or samples containing these nucleic acids. Preferably such detection is for diagnosis, more preferable for the diagosis of a disease, most preferably for the diagnosis of a disease related or linked to the present or abundance of *S. agalactiae*.

Eukaryotes (herein also "individual(s)"), particularly mammals, and especially humans, infected with *S. agalactiae* may be detected at the DNA level by a variety of techniques. By selecting regions of nucleic acids that vary among strains of *S. agalactiae*, preferred candidates for distinguishing a specific strain of *S. agalactiae* can be obtained. Furthermore, by selecting regions of nucleic acids that vary between *S. agalactiae* and other organisms, preferred candidates for distinguishing a *S. agalactiae* from other organisms can be obtained. Nucleic acids for diagnosis may be obtained from an infected individual's cells and tissues, such as bone, blood, muscle, cartilage, and skin. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki et al., *Nature*, 324: 163-166 (1986) prior to analysis. RNA or cDNA may also be used in the same ways. As an example, PCR primers complementary to the nucleic acid forming part of the polynucleotide or the present invention can be used to identify and analyze for its presence and/or expression. Using PCR, characterization of the strain of *S. agalactiae* present in a mammal, and especially a human, may be made by an analysis of the genotype of the prokaryote gene. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the genotype of a reference sequence. Point mutations can be identified by hybridising amplified DNA to radiolabeled RNA or alternatively, radiolabeled antisense DNA sequences. Perfectly matched sequences can be distinguished form mismatched duplexes by Rnase A digestion or by differences in melting temperatures.

Sequence differences between a reference gene and genes having mutations also may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a sequencing primer can be used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic characterization based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualised by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in

the gel at different positions according to their specific melting or partial melting temperatures (see, e.g. Myers et al., *Science,* 230: 1242 (1985)).

Sequence changes at specific locations also may be revealed by nuclease protection assays, such as Rnase and S1 protection or the chemical cleavage method (e. g., Cotton et al., *Proc. Natl. Acad. Sci.,* USA, 85: 4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, Rnase protection, chemical cleavage, direct DNA sequencing or the use fo restriction enzymes, e. g., restriction fragment length polymorphisms (RFLP) and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations also can be detected by *in situ* analysis.

Cells carrying mutations or polymorphisms in the gene of the present invention may also be detected at the DNA level by a variety of techniques, to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations. It is particularly preferred to use RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA or cDNA may also be used for the same purpose, PCR or RT-PCR. As an example, PCR primers complementary to the nucleic acid encoding the polypeptide of the present invention can be used to identify and analyse mutations. The primers may be used to amplify the gene isolated from the individual such that the gene may then be subject to various techniques for elucidation of the DNA sequence. In this way, mutations in the DNA sequence may be diagnosed.

The invention provides a process for diagnosing disease, arising from infection with *S. agalactiae*, comprising determining from a sample isolated or derived from an individual an increased level of expression of a polynucleotide having the sequence of a polynucleotide set forth in the Sequence Listing. Expression of polynucleotide can be measured using any one of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, Rnase protection, Northern blotting, other hybridisation methods and the arrays described herein.

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Cells can be genetically engineered to incorporate polynucleotides and express polypeptides of the present invention. Introduction of a polynucleotides into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., BASIC METHODS IN MOLECULAR BIOLOGY, (1986) and Sambrook et al., MOLECULAR CLONONG: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Polynucleotide constructs in cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector, a single or double-stranded RNA or DNA viral vector. Plasmids generally are designated herein by a lower case p preceded and/or followed by capital letters and/or number, in accordance with standard naming conventions that are familiar to those of skill in the art. Starting plasmids disclosed herein are either commercially available, publicly available, or can be constructed from available plasmids by routine application of well known, published procedures, given the teachings herein. Many plasmids and other cloning and

expression vectors that can be used in accordance with the present invention are well known and readily available to those of skill in the art.

Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise *cis*-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate *trans*-acting factors either are supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

In certain preferred embodiments in this regard, the vectors provide for specific expression. Such specific expression may be inducible expression or expression only in certain types of cells or both inducible and cell-specific. Particularly preferred among inducible vectors are vectors that can be induced for expression by environmental factors that are easy to manipulate, such as temperature and nutrient additives. A variety of vectors suitable to this aspect of the invention, including constitutive and inducible expression vectors for use in prokaryotic and eukaryotic cells, are well known and employed routinely by those of skill in the art.

A great variety of expression vectors can be used to express a polypeptide of the invention. Such vectors include, among other, chromosomal, episomal and virus-derived vectors, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such asbaculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids, all may be used for experssion in accordance with this aspect of the present invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide in a host may be used for expression in this regard.

The appropriate DNA sequence may be inserted into the vector by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s), including, for instance, a promoter to direct mRNA transcription. Representatives of such promoters include, but are not limited to, the phage lambda PL promoter, the *E.coli* lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs.

In general, expression constructs will contain sites for transcription initiation and termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will include a translation initiating AUG or others such as GUG and UUG at the beginning and a terminationcodon appropriately positioned at the end of the polypeptide to be translated.

In addition, the constructs may contain control regions that regulate as well as engender expression. Generally, in accordance with many commonly practiced procedures, such regions will operate by controlling transcription, such as transcription factors, repressor binding sites and termination, among other.

Vectors for propagation and expression generally will include selectable markers and amplification regions, such as, for example, those set forth in Sambrook et al., MOLECULAR CLONONG, A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)

Representative examples of appropriate cells which host said vectors include bacterial cells, such as streptococci, staphylococci, *E. coli*, streptomyces and *Bacillus subtilis cells*; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells.

The following vectors, which are commercially available, are provided by way of example. Among vectors preferred for use in bacteria are pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, Pkk233-3, pDR540, pRIT5 available from Pharmacia, and pBR322 (ATCC 37017). Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, PXT1 and pSG available from Stratagene; and pSVK3,

pBPV, pMSG and pSVL available from Pharmacia. These vectors are listed solely by way of illustration of the many commercially available and well known vectors that are available to those of skill in the art for use in accordance with this aspect of the present invention. It will be appreciated that any other plasmid or vector suitable for, for example, introduction, maintenance, propagation or expression of a polynucleotide or polypeptide of the invention in a host may be used in this aspect of the invention.

Promoter regions can be selected from any desired gene using vectors that contain a reporter transcription unit lacking a promoter region, such as a chloramphenicol acetyl transferase ("CAT") transcription unit, downstream of restriction site or sites for introducing a candidate promoter fragment; i.e., a fragment that may contain a promoter. As is well known, introduction into the vector of a promoter-containing fragment at the restriction site upstream of the cat gene engenders production of CAT activity, which can be detected by standard CAT assays. Vectors suitable to this end are well known and readily available, such as pKK232-8 and pCM7. Promoters for expression of polynucleotides of the present invention include not only well known and readily available promoters, but also promoters that readily may be obtained by the foregoing technique, using a reporter gene.

Among known prokaryotic promoters suitable for expression of polynucleotides and polypeptides in accordance with the present invention are the *E.coli* lacl and lacZ and promoters, the T3 and T7 promoters, the *gpt* promoter, the lambda PR, PL promoters and the trp promoter.

Among known eukaryotic promoters suitable in this regard are the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus "(RSV"), and metallothionein promoters, such as the mouse metallothionein-I promoter.

Recombinant expression vectors will include, for example, origins of replication, a promoter preferably derived from a highly-expressed gene to direct transcription of a downstream structural sequence, and a selectable marker to permit isolation of vector containing cells after exposure to the vector.

Polynucleotides of the invention, encoding the heterologous structural sequence of a polypeptide of the invention generally will be inserted into the vector using standard techniques so that it is operably linked to the promoter for expression. The polynucleotide will be positioned so that the transcription start site will be 5' to the AUG that initiates translation of the polypeptide to be expressed. Generally, there will be no other open reading frames that begin with an initiation codon, usually AUG, and lie between the ribosome binding site and the initiationcodon. Also, generally, there will be a translation stop codon at the end of the polypeptide and there will be a polyacenylation signal in constructs for use in eukaryotic hosts. Transcription termination signal appropriately disposed at the 3'end of the transcribed region may also be included in the polynucleotide construct.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide.

These signals may be endogenous to the polypeptide or they may be heterologous signals.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N- or C-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, regions also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability or to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize or purify polypeptides. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another protein or part thereof. In drug discovery, for example, proteins have been fused with antibody Fc portions for the purpose of high-throughout screening assays to identify antagonists. See, D. Bennett *et al., Journal of Molecular Recognition,* Vol. 8 52-58 (1995) and K. Johanson *et al., The Journal of Biological Chemistry,* Vol. 270, No.16, pp 9459-9471 (1995).

Cells typically then are harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents; such methods are well known to those skilled in the art.

Mammalian expression vectors may comprise expression sequences, such as an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation regions, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences that are useful or necessary for expression.

The polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

The polypeptides according to the present invention can be produced by chemical synthesis as well as by biotechnological means. The latter comprise the transfection or transformation of a host cell with a vector containing a nucleic acid according to the present invention and the cultivation of the transfected or transformed host cell under conditions which are known to the ones skilled in the art. The production method may also comprise a purification step in order to purify or isolate the polypeptide to be manufactured. In a preferred embodiment the vector is a vector according to the present invention.

In a further aspect the present invention relates to an antibody directed to any of the polypeptides, derivatives or fragments thereof according to the present invention. The present invention includes, for example, monoclonal and polyclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of a Fab expression

library. It is within the present invention that the antibody may be chimeric, i. e. that different parts thereof stem from different species or at least the respective sequences are taken from different species.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a non-human. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique known in the art which provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Köhler, G. and Milstein, C., *Nature 256*: 495-497 (1975); Kozbor *et al., Immunology Today 4:* 72 (1983); Cole et al., pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY,* Alan R. Liss, Inc. (1985); U.S. Patent No. 5,545,403; U.S. Patent No. 5,545,405; U.S. Patent No. 5,654,403; U.S. Patent No. 5,792,838; U.S. Patent No. 5,316,938; U. S. Patent No. 5,633,162; U.S. Patent No. 5,644,036; U.S. Patent No. 5,858,725.

Techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

Alternatively, phage display technology could be utilized to select antibody genes with binding activities towards the polypeptide either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-Fbp or from naïve libraries (McCafferty, J. et al., (1990), Nature 348, 552-554; Marks, J. et al., (1992) Biotechnology 10, 779-783). The affinity of these antibodies can also be improved by chain shuffling (Clackson, T. et al., (1991) Nature 352, 624-628).

If two antigen binding domains are present, each domain may be directed against a different epitope – termed 'bispecific' antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or purify the polypeptide of the present invention by attachment of the antibody to a solid support for isolation and/or purification by affinity chromatography.

Thus, among others, antibodies against the polypeptide of the present invention may be employed to inhibit and/or treat infections, particularly bacterial infections and especially infections arising from *S. agalactiae.*

Polypeptide derivatives include antigenically, epitopically or immunologically equivalent derivatives which form a particular aspect of this invention. The term "antigenically equivalent derivative" as used herein encompasses a polypeptide or its equivalent which will be specifically recognized by certain antibodies which, when raised to the protein or polypeptide according to the present invention, interfere with the immediate physical interaction between pathogen and mammalian host. The term "immunologically equivalent derivative" as used herein encompasses a peptide or its equivalent which when used in a suitable formulation to raise antibodies in a vertebrate, the antibodies act to interfere with the immediate physical interaction between pathogen and mammalian host.

The polypeptide, such as an antigenically or immunologically equivalent derivative or a fusion protein thereof can be used as an antigen to immunize a mouse or other animal such as a rat or chicken. The fusion protein may provide stability to the polypeptide. The antigen may be associated, for example by conjugation, with an immunogenic carrier protein, for example bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). Alternatively, a multiple antigenic peptide comprising multiple copies of the protein or polypeptide, or an antigenically or immunologically equivalent polypeptide thereof, may be sufficiently antigenic to improve immunogenicity so as to obviate the use of a carrier.

Preferably the antibody or derivative thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized", wherein the complimentarity determining region(s) of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described

in Jones, P. et al. (1986), Nature 321, 522-525 or Tempest at al., (1991) Biotechnology 9, 266-273.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscle (Wolff et al., Hum Mol Genet 1992, 1:363, Manthorpe et al., Hum. Gene Ther. 1963:4, 419), delivery of DNA complexed with specific protein carriers (Wu et al., J Biol Chem 1989:264, 16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, PNAS, 1986:83, 9551), encapsulation of DNA in various forms of liposomes (Kaneda et al., Science 1989:243, 375), particle bombardment (Tang et al., Nature 1992, 356:152, Eisenbraun et al., DNA Cell Biol 1993, 12:791) and *in vivo* infection using cloned retroviral vectors (Seeger et al., PNAS 1984:81, 5849).

In a further aspect the present invention relates to a peptide binding to any of the polypeptides according to the present invention, and a method for the manufacture of such peptides whereby the method is characterized by the use of the polypeptides according to the present invention and the basic steps are known to the one skilled in the art.

Such peptides may be generated by using methods according to the state of the art such as phage display or ribosome display. In case of phage display, basically a library of peptide is generated, such as in form of phages, and this kind of libraries is contacted with the target molecule, in the present case the polypeptides according to the present invention. Those peptides binding to the target molecule are subsequently removed, preferably as a complex with the target molecule, from the respective reaction. It is known to the one skilled in the art that the binding characteristics, at least to a certain extend, depend on the particularly realized experimental set-up such as the salt concentration and the like. After separating those peptides binding to the target molecule with a higher affinity or a bigger force, from the non-binding members of the library, and optionally also after removal of the target molecule from the complex of target molecule and peptide, the respective peptide(s) may subsequently be characterised. Prior to the characterisation optionally an amplification step is realized such as, e. g. by propagating the peptide coding phages. The characterisation preferably comprises the sequencing of the target binding peptides. Basically, the peptides are not limited in their lengths, however, preferably peptides having a lengths from about 8 to 20 amino acids are

preferably obtained in the respective methods. The size of the libraries may be about 10^2 to 10^{18}, preferably 10^8 to 10^{15} different peptides, however, is not limited thereto.

A particular form of target binding polypeptides are the so-called "anticalines" which are, among others, described in German patent application DE 197 42 706.

In a further aspect the present invention relates to functional nucleic acids interacting with any of the polypeptides according to the present invention, and a method for the manufacture of such functional nucleic acids whereby the method is characterized by the use of the polypeptides according to the present invention and the basic steps are known to the one skilled in the art.. The functional nucleic acids are preferably aptamers and spiegelmers.

Aptamers are D-nucleic acids which are either single stranded or double stranded and which specifically interact with a target molecule. The manufacture or selection of aptamers is, e. g., described in European patent EP 0 533 838. Basically the following steps are realized. First, a mixture of nucleic acids, i. e. potential aptamers, is provided whereby each nucleic acid typically comprises a segment of several, preferably at least eight subsequent randomised nucleotides. This mixture is subsequently contacted with the target molecule whereby the nucleic acid(s) bind to the target molecule, such as based on an increased affinity towards the target or with a bigger force thereto, compared to the candidate mixture. The binding nucleic acid(s) are/is subsequently separated from the remainder of the mixture. Optionally, the thus obtained nucleic acid(s) is amplified using, e. g. polymerase chain reaction. These steps may be repeated several times giving at the end a mixture having an increased ratio of nucleic acids specifically binding to the target from which the final binding nucleic acid is then optionally selected. These specifically binding nucleic acid(s) are referred to aptamers. It is obvious that at any stage of the method for the generation or identification of the aptamers samples of the mixture of individual nucleic acids may be taken to determine the sequence thereof using standard techniques. It is within the present invention that the aptamers may be stabilized such as, e. g., by introducing defined chemical groups which are known to the one skilled in the art of generating aptamers. Such modification may for example reside in the introduction of an amino group at the 2'-position of the sugar moiety of the nucleotides. Aptamers are currently used as therapeutical agens. However, it is also within the present invention that the thus selected or generated aptamers may be used for target validation and/or as lead substance for the development of medicaments, preferably of medicaments based on

small molecules. This is actually done by a competition assay whereby the specific interaction between the target molecule and the aptamer is inhibited by a candidate drug whereby upon replacement of the aptamer from the complex of target and aptamer it may be assumed that the respective drug candidate allows a specific inhibition of the interaction between target and aptamer, and if the interaction is specific, said candidate drug will, at least in principle, be suitable to block the target and thus decrease its biological availability or activity in a respective system comprising such target. The thus obtained small molecule may then be subject to further derivatisation and modification to optimise its physical, chemical, biological and/or medical characteristics such as toxicity, specificity, biodegradability and bioavailability.

Spiegelmers and their generation or manufacture is based on a similar principle. The manufacture of spiegelmers is described in international patent application WO 98/08856. Spiegelmers are L-nucleic acids, which means that they are composed of L-nucleotides rather than D-nucleotides as aptamers are. Spiegelmers are characterized by the fact that they have a very high stability in biological system and, comparable to aptamers, specifically interact with the target molecule against which they are directed. In the process of generating spiegelmers, a heterogonous population of D-nucleic acids is created and this population is contacted with the optical antipode of the target molecule, in the present case for example with the D-enantiomer of the naturally occurring L-enantiomer of the polypeptides according to the present invention. Subsequently, those D-nucleic acids are separated which do not interact with the optical antipode of the target molecule. But those D-nucleic acids interacting with the optical antipode of the target molecule are separated, optionally determined and/or sequenced and subsequently the corresponding L-nucleic acids are synthesized based on the nucleic acid sequence information obtained from the D-nucleic acids. These L-nucleic acids which are identical in terms of sequence with the aforementioned D-nucleic acids interacting with the optical antipode of the target molecule, will specifically interact with the naturally occurring target molecule rather than with the optical antipode thereof. Similar to the method for the generation of aptamers it is also possible to repeat the various steps several times and thus to enrich those nucleic acids specifically interacting with the optical antipode of the target molecule.

In a further aspect the present invention relates to functional nucleic acids interacting with any of the nucleic acid molecules according to the present invention, and a method for the

manufacture of such functional nucleic acids whereby the method is characterized by the use of the nucleic acid molecules and their respective sequences according to the present invention and the basic steps are known to the one skilled in the art. The functional nucleic acids are preferably ribozymes, antisense oligonucleotides and siRNA.

Ribozymes are catalytically active nucleic acids which preferably consist of RNA which basically comprises two moieties. The first moiety shows a catalytic activity whereas the second moiety is responsible for the specific interaction with the target nucleic acid, in the present case the nucleic acid coding for the polypeptides according to the present invention. Upon interaction between the target nucleic acid and the second moiety of the ribozyme, typically by hybridisation and Watson-Crick base pairing of essentially complementary stretches of bases on the two hybridising strands, the catalytically active moiety may become active which means that it catalyses, either intramolecularly or intermolecularly, the target nucleic acid in case the catalytic activity of the ribozyme is a phosphodiesterase activity. Subsequently, there may be a further degradation of the target nucleic acid which in the end results in the degradation of the target nucleic acid as well as the protein derived from the said target nucleic acid. Ribozymes, their use and design principles are known to the one skilled in the art, and, for example described in Doherty and Doudna (Ribozym structures and mechanism. Annu ref. Biophys. Biomolstruct. 2001 ; 30 :457-75) and Lewin and Hauswirth (Ribozyme Gene Therapy: Applications for molecular medicine. 2001 7: 221-8).

The activity and design of antisense oligonucleotides for the manufacture of a medicament and as a diagnostic agent, respectively, is based on a similar mode of action. Basically, antisense oligonucleotides hybridise based on base complementarity, with a target RNA, preferably with a mRNA, thereby activate RNase H. RNase H is activated by both phosphodiester and phosphorothioate-coupled DNA. Phosphodiester-coupled DNA, however, is rapidly degraded by cellular nucleases with the exception of phosphorothioate-coupled DNA. These resistant, non-naturally occurring DNA derivatives do not inhibit RNase H upon hybridisation with RNA. In other words, antisense polynucleotides are only effective as DNA RNA hybride complexes. Examples for this kind of antisense oligonucleotides are described, among others, in US-patent US 5,849,902 and US 5,989,912. In other words, based on the nucleic acid sequence of the target molecule which in the present case are the nucleic acid molecules for the polypeptides according to the present invention, either from the target protein from which a respective nucleic acid sequence may in principle be deduced, or by

knowing the nucleic acid sequence as such, particularly the mRNA, suitable antisense oligonucleotides may be designed base on the principle of base complementarity.

Particularly preferred are antisense-oligonucleotides which have a short stretch of phosphorothioate DNA (3 to 9 bases). A minimum of 3 DNA bases is required for activation of bacterial RNase H and a minimum of 5 bases is required for mammalian RNase H activation. In these chimeric oligonucleotides there is a central region that forms a substrate for RNase H that is flanked by hybridising "arms" comprised of modified nucleotides that do not form substrates for RNase H. The hybridising arms of the chimeric oligonucleotides may be modified such as by 2′-O-methyl or 2′-fluoro. Alternative approaches used methylphosphonate or phosphoramidate linkages in said arms. Further embodiments of the antisense oligonucleotide useful in the practice of the present invention are P-methoxyoligonucleotides, partial P-methoxyoligodeoxyribonucleotides or P-methoxyoligonucleotides.

Of particular relevance and usefulness for the present invention are those antisense oligonucleotides as more particularly described in the above two mentioned US patents. These oligonucleotides contain no naturally occurring 5′→3′-linked nucleotides. Rather the oligonucleotides have two types of nucleotides: 2′-deoxyphosphorothioate, which activate RNase H, and 2′-modified nucleotides, which do not. The linkages between the 2′-modified nucleotides can be phosphodiesters, phosphorothioate or P-ethoxyphosphodiester. Activation of RNase H is accomplished by a contiguous RNase H-activating region, which contains between 3 and 5 2′-deoxyphosphorothioate nucleotides to activate bacterial RNase H and between 5 and 10 2′- deoxyphosphorothioate nucleotides to activate eucaryotic and, particularly, mammalian RNase H. Protection from degradation is accomplished by making the 5′ and 3′ terminal bases highly nuclease resistant and, optionally, by placing a 3′ terminal blocking group.

More particularly, the antisense oligonucleotide comprises a 5′ terminus and a 3′ terminus; and from 11 to 59 5′→3′-linked nucleotides independently selected from the group consisting of 2′-modified phosphodiester nucleotides and 2′-modified P-alkyloxyphosphotriester nucleotides; and wherein the 5′-terminal nucleoside is attached to an RNase H-activating region of between three and ten contiguous phosphorothioate-linked deoxyribonucleotides, and wherein the 3′-terminus of said oligonucleotide is selected from the group consisting of

an inverted deoxyribonucleotide, a contiguous stretch of one to three phosphorothioate 2′-modified ribonucleotides, a biotin group and a P-alkyloxyphosphotriester nucleotide.

Also an antisense oligonucleotide may be used wherein not the 5′ terminal nucleoside is attached to an RNase H-activating region but the 3′ terminal nucleoside as specified above. Also, the 5′ terminus is selected from the particular group rather than the 3′ terminus of said oligonucleotide.

The nucleic acids as well as the polypeptides according to the present invention may be used as or for the manufacture of vaccines. Preferably such vaccine is for the prevention or treatment of diseases caused by, related to or associated with GBS. In so far another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal, which comprises inoculating the individual with the polypeptide of the invention, or a fragment or variant thereof, adequate to produce antibody to protect said individual from infection, particularly bacterial infection and most particularly Streptococcus infections.

Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises, through gene therapy or otherwise, delivering a nucleic acid functionally encoding the polypeptide, or a fragment or a variant thereof, for expressing the polypeptide, or a fragment or a variant thereof *in vivo* in order to induce an immunological response to produce antibodies or a cell mediated T cell response, either cytokine-producing T cells or cytotoxic T cells, to protect said individual from disease, whether that disease is already established within the individual or not. One way of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise.

A further aspect of the invention relates to an immunological composition which, when introduced into a host capable of having induced within it an immunological response, induces an immunological response in such host, wherein the composition comprises recombinant DNA which codes for and expresses an antigen of the polypeptide of the present invention. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity or cellular immunity such as that arising from CTL or CD4+ T cells.

The polypeptide of the invention or a fragment thereof may be fused with co-protein which may not by itself produce antibodies, but is capable of stabilizing the first protein and producing a fused protein which will have immunogenic and protective properties. This fused recombinant protein preferably further comprises an antigenic co-protein, such as Glutathione-S-transferase (GST) or beta-galactosidase, relatively large co-proteins which solubilise the protein and facilitate production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system. The co-protein may be attached to either the amino or carboxy terminus of the first protein.

Provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides or polynucleotides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. et al., Science 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof which have been shown to encode non-variable regions of bacterial cell surface proteins in DNA constructs used in such genetic immunization experiments in animal models of infection with *S. agalactiae*. Such fragments will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. This approach can allow for the subsequent preparation of monoclonal antibodies of particular value from the requisite organ of the animal successfully resisting or clearing infection for the development of prophylactic agents or therapeutic treatments of *S. agalactiae* infection in mammals, particularly humans.

The polypeptide may be used as an antigen for vaccination of a host to produce specific antibodies which protect against invasion of bacteria, for example by blocking adherence of bacteria to damaged tissue. Examples of tissue damage include wounds in skin or connective tissue caused e.g. by mechanical, chemical or thermal damage or by implantation of indwelling devices, or wounds in the mucous membranes, such as the mouth, mammary glands, urethra or vagina.

The present invention also includes a vaccine formulation which comprises the immunogenic recombinant protein together with a suitable carrier. Since the protein may be broken down in the stomach, it is preferably administered parenterally, including, for example, administration

that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials, and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in-water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

It is also within the present invention that the vaccine comprises apart from the polypeptide and/or nucleic acid molecule according to the present invention other compounds which are biologically or pharmaceutically active. Preferably, the vaccine composition comprises at least one polycationic peptide. The polycationic compound(s) to be used according to the present invention may be any polycationic compound which shows the characteristic effects according to the WO 97/30721. Preferred polycationic compounds are selected from basic polypeptides, organic polycations, basic polyamino acids or mixtures thereof. These polyamino acids should have a chain length of at least 4 amino acid residues (WO 97/30721). Especially preferred are substances like polylysine, polyarginine and polypeptides containing more than 20 %, especially more than 50 % of basic amino acids in a range of more than 8, especially more than 20, amino acid residues or mixtures thereof. Other preferred polycations and their pharmaceutical compositions are described in WO 97/30721 (e. g. polyethyleneimine) and WO 99/38528. Preferably these polypeptides contain between 20 and 500 amino acid residues, especially between 30 and 200 residues.

These polycationic compounds may be produced chemically or recombinantly or may be derived from natural sources.

Cationic (poly)peptides may also be anti-microbial with properties as reviewed in Ganz et al., 1999; Hancock, 1999. These (poly)peptides may be of prokaryotic or animal or plant origin or may be produced chemically or recombinantly (WO 02/13857). Peptides may also belong to

the class of defensins (WO 02/13857). Sequences of such peptides can be, for example, be found in the Antimicrobial Sequences Database under the following internet address:

http://www.bbcm.univ.trieste.it/~tossi/pag2.html

Such host defence peptides or defensives are also a preferred form of the polycationic polymer according to the present invention. Generally, a compound allowing as an end product activation (or down-regulation) of the adaptive immune system, preferably mediated by APCs (including dendritic cells) is used as polycationic polymer.

Especially preferred for use as polycationic substances in the present invention are cathelicidin derived antimicrobial peptides or derivatives thereof (International patent application WO 02/13857, incorporated herein by reference), especially antimicrobial peptides derived from mammal cathelicidin, preferably from human, bovine or mouse.

Polycationic compounds derived from natural sources include HIV-REV or HIV-TAT (derived cationic peptides, antennapedia peptides, chitosan or other derivatives of chitin) or other peptides derived from these peptides or proteins by biochemical or recombinant production. Other preferred polycationic compounds are cathelin or related or derived substances from cathelin. For example, mouse cathelin is a peptide which has the amino acid sequence NH_2-RLAGLLRKGGEKIGEKLKKIGOKIKNFFQKLVPQPE-COOH. Related or derived cathelin substances contain the whole or parts of the cathelin sequence with at least 15-20 amino acid residues. Derivations may include the substitution or modification of the natural amino acids by amino acids which are not among the 20 standard amino acids. Moreover, further cationic residues may be introduced into such cathelin molecules. These cathelin molecules are preferred to be combined with the antigen. These cathelin molecules surprisingly have turned out to be also effective as an adjuvant for a antigen without the addition of further adjuvants. It is therefore possible to use such cathelin molecules as efficient adjuvants in vaccine formulations with or without further immunactivating substances.

Another preferred polycationic substance to be used according to the present invention is a synthetic peptide containing at least 2 KLK-motifs separated by a linker of 3 to 7 hydrophobic

amino acids (International patent application WO 02/32451, incorporated herein by reference).

The pharmaceutical composition of the present invention may further comprise Immunostimulatory nucleic acid(s). Immunostimulatory nucleic acids are e. g. neutral or artificial CpG containing nucleic acid, short stretches of nucleic acid derived from non-vertebrates or in form of short oligonucleotides (ODNs) containing non-methylated cytosine-guanine di-nucleotides (CpG) in a certain base context (e.g. described in WO 96/02555) Alternatively, also nucleic acids based on inosine and cytidine as e.g. described in the WO 01/93903, or deoxynucleic acids containing deoxy-inosine and/or deoxyuridine residues (described in WO 01/93905 and PCT/EP 02/05448, incorporated herein by reference) may preferably be used as immunostimulatory nucleic acids for the present invention. Preferablly, the mixtures of different immunostimulatory nucleic acids may be used according to the present invention.

It is also within the present invention that any of the aforementioned polycationic compounds is combined with any of the immunostimulatory nucleic acids as aforementioned. Preferably, such combinations are according to the ones as described in WO 01/93905, WO 02/32451, WO 01/54720, WO 01/93903, WO 02/13857 and PCT/EP 02/05448 and the Austrian patent application A 1924/2001, incorporated herein by reference.

In addition or alternatively such vaccine composition may comprise apart from the polypeptide/nucleic acid molecules according to the present invention a neuroactive compound. Preferably, the neuroactive compound is human growth factor as, e.g. described in WO 01/24822. Also preferably, the neuroactive compound is combined with any of the polycationic compounds and/or immunostimulatory nucleic acids as afore-mentioned.

In a further aspect the present invention is related to a pharmaceutical composition. Such pharmaceutical composition is, for example, the vaccine described herein. Also a pharmaceutical composition is a pharmaceutical composition which comprises any of the following compounds or combinations threreof: the nucleic acids according to the present invention, the polypeptides according to the present invention, the vector according to the present invention, the cells according to the present invention, the antibody according to the present invention, the functional nucleic acids according to the present invention and the

binding peptides such as the anticalines according to the present invention, any agonists and antagonists screened as described herein. In connection therewith any of these compounds may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

The pharmaceutical compositions generally are administered in an amount effective for treatment or prophylaxis of a specific indication or indications. In general, the compositions are administered in an amount of active agent of at least about 10 µg/kg body weight. In most cases they will be administered in one or more doses in an amount not in excess of about 8 mg/kg body weight per day. Preferably, in most cases, dose is from about 10 µg/kg to about 1 mg/kg body weight, daily. For administration particularly to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg and typically around 1 mg/kg. For example, a dose may be 1 mg/kg daily. It will be appreciated that optimum dosage will be determined by standard methods for each treatment modality and indication, taking into account the indication, its severity, route of administration, complicating conditions and the like. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition may be formulated for topical application, for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1 % to about 98 % by weight of the formulation; more usually they will constitute up to about 80 % by weight of the formulation.

The pharmaceutical composition may be administered in conjunction with an in-dwelling device. In-dwelling devices include surgical implants, prosthetic devices and catheters, i. e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, peacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters, etc.

The composition of the invention may be administered by injection to achieve a systematic effect against relevant bacteria shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent Streptococcus infections.

Many orthopaedic surgeons consider that humans with prosthetic joints should be considered for antibiotic prophylaxis before dental treatment that could produce a bacteremia. Late deep infection is serious complication sometimes leading to loss of the prosthetic joint and is accompanied by significant morbidity and mortality. It may therefore be possible to extend the use of the active agent as a replacement for prophylactic antibiotics in this situation.

In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

Alternatively, the composition of the invention may be used to bathe an indwelling device immediately before insertion. The active agent will preferably be present at a concentration of 1 µg/ml to 10 mg/ml for bathing of wounds or indwelling devices.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 µg/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks.

With the indicated dose range, no adverse toxicological effects should be observed with the compounds of the invention which would preclude their administration to suitable individuals.

The antibodies described above may also be used as diagnostic reagents to detect the presence of bacteria containing the polypeptides according to the present invention.

In a further embodiment the present invention relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. The ingredient(s) can be present in a useful amount, dosage, formulation or combination. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, reflecting approval by the agency of the manufacture, use or sale of the product for human administration.

In connection with the present invention any disease related use as disclosed herein such as, e. g. use of the pharmaceutical composition or vaccine, is particularly a disease or diseased condition which is caused, linked or associated with Gram-positive bacteria, more particularly bacteria selected from the group comprising Streptococci, Staphylococci and Lactococci. More preferably, the microorganisms are selected from the group comprising *S. agalactiae*, *S. pyogenes*, *S. pneumoniae* and *S. mutans*. In connection therewith it is to be noted that *S. agalactiae* comprises several strains including those disclosed herein. Also, the disease may be particularly a disease occurring in any patient selected from the group comprising people with chronic illness such as diabetes mellitus and liver failure, pregnant women, the fetus and the newborn. A disease related, caused or associated with the bacterial infection to be prevented and/or treated according to the present invention includes in neonates sepsis,

pneumonia and meningitis, and in adults sepsis and soft tissue infections. Pregnancy-related infections are sepsis, amnionitis, urinary tract infection and stillbirth.

In a still further embodiment the present invention is related to a screening method using any of the polypeptides or nucleic acids according to the present invention. Screening methods as such are known to the one skilled in the art and can be designed such that an agonist or an antagonist is screened. Preferably an antagonist is screened which in the present case inhibits or prevents the binding of any polypeptide according to the present invention to an interaction partner. Such interaction partner can be a naturally occurring interaction partner or a non-naturally occurring interaction partner. Preferable the interaction partner is fibrinogen or a fragment thereof in case of FbsA or any host cell in case of PabA, PabB, PabC, and PabD, including epithelial cells, preferably human epithelial cells.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the function of polypeptides or polynucleotides of the present invention, such as its interaction with a binding molecule. The method of screening may involve high-throughput.

For example, to screen for agonists or antagonists, the interaction partner of the polynucleotide and nucleic acid, respectively, according to the present invention, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, may be prepared from a cell that expresses a molecule that binds to the polypeptide of the present invention. The preparation is incubated with labelled polypeptide in the absence or the presence of a candidate molecule which may be an agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labelled ligand. Molecules which bind gratuitously, i. e., without inducing the functional effects of the polypeptide, are most likely to be good antagonists. Molecules that bind well and elicit functional effects that are the same as or closely related to the polypeptide are good agonists.

The functional effects of potential agonists and antagonists may by measured, for instance, by determining activity of a reporter system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of the polypeptide of the present invention or molecules that elicit the same effects as the polypeptide. Reporter

systems that may be useful in the regard include but are not limited to colorimetric labelled substrate converted into product, a reporter gene that is responsive to changes in the functional activity of the polypeptide, and binding assays known in the art.

Another example of an assay for antagonists is a competitive assay that combines the polypeptide of the present invention and a potential antagonist with membrane-bound binding molecules, recombinant binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. The polypeptide can be labelled such as by radioactivity or a colorimetric compound, such that the number of polypeptide molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polypeptide of the invention and thereby inhibit or extinguish its acitivity. Potential antagonists also may be small organic molecules, a peptide , a polypeptide such as a closely related protein or antibody that binds to the same sites on a binding molecule without inducing functional activity of the polypeptide of the invention.

Potential antagonists include a small molecule which binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules.

Other potential antagonists include antisense molecules (see Okano, J. *Neurochem.* 56:560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION;* CRC Press, Boca Raton, FL (1988), for a description of these molecules).

Preferred potential antagonists include derivatives of the polypeptides of the invention.

As used herein the activity of a polypeptide according to the present invention is its capability to bind to any of its interaction partner or the extent of such capability or its binding to its or any interaction partner.

In a particular aspect, the invention provides the use of the polypeptide, polynucleotide or inhibitor of the invention to interfere with the initial physical interaction between a pathogen and mammalian host responsible for sequelae of infection. In particular the molecules of the invention may be used: i) in the prevention of adhesion of *S. agalactiae* to mammalian extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; ii) to block protein mediated mammalian cell invasion by, for example, initiating phosphorylation of mammalian tyrosine kinases (Rosenshire et al., *Infect. Immun.* 60:2211 (1992)). iii) to block bacterial adhesion between mammalian extracellular matrix proteins and bacterial proteins which mediate tissue damage; iv) to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

Each of the DNA coding sequence provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein upon expression can be used as a target for the screening of antibacterial drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The antagonists and agonists may be employed, for instance, to inhibit diseases arising from infection with Streptococcus, especially *S. agalactiae*, such as sepsis.

In a still further aspect the present invention is related to an affinity device such affinity device comprises as least a support material and any of the polypeptides according to the present invention which is attached to the support material. Because of the specificity of the polypeptides according to the present invention for their target cells or target molecules or their interaction partners, the polypeptides allow a selective removal of their interaction partner(s) from any kind of sample applied to the support material provided that the conditions for a binding are met. The sample may by a biological or medical sample, including but not limited to, fermentation broth, cell debris, cell preparation, tissue preparation, organ preparation, blood, urine, lymph liquid, liquor and the like.

The polypeptide may be attached to the matrix in a covalent or non-covalent manner. Suitable support material is known to the one skilled in the art and can be selected from the group comprising cellulose, silicon, glass, aluminium, paramagnetic beads, starch and dextrane.

The present invention is further illustrated by the following figures, examples and the sequence listing from which further features, embodiments, and advantages may be taken. It is to be understood that the present examples are give by way of illustration only and not by way of limitation of the disclosure.

In connection with the present invention

Fig. 1 shows the DNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the serotype III GBS strain 6313;

Fig. 2 the result of a Southern Blot analysis;

Fig. 3 the DNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the serotype Ia GBS strain 706 S2;

Fig. 4 the DNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the serotype Ib GBS strain 33H1A;

Fig. 5 DNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the serotype II GBS strain 176 H4A;

Fig. 6 DNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the capsule GBS mutant O90R;

Fig. 7 DNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the serotype V GBS strain SS1169;

Fig. 8 a schematic comparison of the FbsA proteins from the GBS strains 6313 (serotype III), 706 S2 (serotype Ia), 33H1A (serotype Ib), O176 H4A (serotype II); O90R (derived from serotype Ia) and SS1169 (serotype V), respectively;

Fig. 9 the result of a western blot analysis of truncated FbsA derivatives to identify the fibrinogen binding domain in FbsA;

Fig. 10 a diagram illustrating the competitive inhibition of fibrinogen binding to GBS 6313 by the purified fusion proteins FbsA-19, FbsA-9 and Bsp, respectively;

Fig. 11 the result of a spot membrane analysis of fibrinogen binding by synthetic peptides derived from the repeat unit of FbsA;

Fig. 12 the result of a spot membrane analysis of the fibrinogen binding repeat unit;

Fig. 13 a diagram illustrating the competitive inhibition of fibrinogen binding to GBS 6313 by synthetic peptides;

Fig. 14 a diagram illustrating eukaryotic cell adherence (A) and invasion (B) of GBS strains 6313, 706 S2, and O90R and their respective *fbsA* deletion mutants;

Fig. 15 the result of a peptide ELISA of FbsA peptides with human sera;

Fig. 16 the DNA sequence of the pabA/B-encoding region and the deduced PabA (nt 319-2964) and PabB (nt3087-5111) proteins from GBS 6313;

Fig. 17 the DNA sequence of the *pabC*/D-encoding region and the deduced PabC (nt 487-2394) and PabD (nt 2461-3006) proteins from GBS 6313;

Fig. 18 a picture from a scanning electron microscopy of A549 cells;

Fig. 19 a diagram illustrating the adherence of GBS 6313 to and invasion of A549 cells in the presence of 100µg/ml of PabA, PabB, PabC or PabD fusion proteins;

Fig. 20 a diagram illurstrating eukaryotic cell adherence and internalization by GBS 6313 and its *pabA* and *pabB* deletion mutants; and

Fig. 21 the result of a Western Blot testing anti-PabA, anti-PabB, and anti-PabD antisera for their sensitivity.

The figures to which it might be referred to in the specification are described in the following in more detail.

Fig. 1 shows the DNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the serotype III GBS strain 6313. The putative ribosomal binding site (RBS) is underlined and the potential transcriptional terminator is indicated by antiparallel arrows. Within the deduced FbsA protein, letters in bold and italic indicate the putative signal peptide sequence and letters in bold and underlined mark the cell wall anchor motif LPKTG. Repeats in FbsA are numbered and marked by arrows.

Fig. 2 shows a southern blot analysis to determine the presence of the *fbsA* gene in different clinical isolates of GBS. Chromosomal DNA from different GBS strains belonging to serotypes Ia, Ib, II, III, IV, and V, respectively was digested with *Hin*dIII and, after size separation and blotting onto nylon membrane, hybridised with a digoxigenin-labelled *fbsA*-specific DNA probe.

Fig. 3 shows the DNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the serotype Ia GBS strain 706 S2. The putative ribosomal binding site (RBS) is underlined and the potential transcriptional terminator is indicated by antiparallel arrows. Within the deduced FbsA protein, letters in bold and italic indicate the putative signal peptide sequence and letters in bold and underlined mark the cell wall anchor motif LPKTG. Repeats in FbsA are numbered and marked by arrows.

Fig. 4 shows the DNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the serotype Ib GBS strain 33H1A. The putative ribosomal binding site (RBS) is underlined and the potential transcriptional terminator is indicated by antiparallel arrows. Within the deduced FbsA protein, letters in bold and italic indicate the putative signal peptide sequence and letters in bold and underlined mark the cell wall anchor motif LPKTG. Repeats in FbsA are numbered and marked by arrows.

Fig. 5 shows the DNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the serotype II GBS strain 176 H4A. The putative ribosomal binding site (RBS) is underlined and the potential transcriptional terminator is indicated by antiparallel arrows. Within the deduced FbsA protein, letters in bold and italic indicate the putative signal peptide sequence and letters in bold and underlined mark the cell wall anchor motif LPKTG. Repeats in FbsA are numbered and marked by arrows.

Fig. 6 shows the DNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the capsule GBS mutant O90R. The putative ribosomal binding site (RBS) is underlined and the potential transcriptional terminator is indicated by antiparallel arrows. Within the deduced FbsA protein, letters in bold and italic indicate the putative signal peptide sequence and letters in bold and underlined mark the cell wall anchor motif LPKTG. Repeats in FbsA are numbered and marked by arrows.

Fig. 7 shows theDNA sequence of the *fbsA*-encoding region and the deduced FbsA protein from the serotype V GBS strain SS1169. The putative ribosomal binding site (RBS) is underlined and the potential transcriptional terminator is indicated by antiparallel arrows. Within the deduced FbsA protein, letters in bold and italic indicate the putative signal peptide sequence and letters in bold and underlined mark the cell wall anchor motif LPKTG. Repeats in FbsA are numbered and marked by arrows.

Fig. 8 shows a schematic comparison of the FbsA proteins from the GBS strains 6313 (serotype III), 706 S2 (serotype Ia), 33H1A (serotype Ib), O176 H4A (serotype II), O90R (derived from serotype Ia) and SS1169 (serotype V), respectively. Indicated are the locations of the signal peptide (black box), the wall-spanning region (WSR; boxes with vertical bars), the cell wall anchor motif (LPKTG), and the membrane-spanning region (MSR; boxes with diagonal bars). The number of individual repeats is indicated for each protein. Grey boxes represent a repeat with the sequence motif 'GNVLERRQRDAENRSQ', boxes with horizontal bars represent repeats with an R14K substitution and dotted boxes show the location of repeats with both an A11V and R14K substitution. Repeats that carry an E12D substitution are indicated below the FbsA proteins from GBS strains 33H1A and SS1169. Above FbsA from 33H1A, a repeat carrying a single A11V substitution is indicated.

Fig. 9 shows a western blot analysis of truncated FbsA derivatives to identify the fibrinogen binding domain in FbsA. Hexahistidyl-tagged fusion proteins, representing the mature FbsA protein (FbsA-19), the N-terminal repeat-containing region (FbsA-N) or the C-terminal part (FbsA-C) of FbsA were separated by SDS-PAGE, blotted onto nitrocellulose and tested for their binding to human fibrinogen. The fibrinogen binding activity of the three proteins encoded by different constructs are indicated below the schematic FbsA drawing.

Fig. 10 shows the competitive inhibition of fibrinogen binding to GBS 6313 by the purified fusion proteins FbsA-19, FbsA-9 and Bsp, respectively. FbsA-9 differs from FbsA-19 in that it contains only 9 repeats in its repeat domain. The binding assay was performed with ^{125}I-labelled fibrinogen in the presence of different concentrations of each fusion protein. Each experiment was performed at least in triplicate.

Fig. 11 shows a spot membrane analysis of fibrinogen binding by synthetic peptides derived from the repeat unit of FbsA. Fibrinogen binding was tested with peptides carrying the FbsA repeat motif 'GNVLERRQRDAENRSQ' (SEQ ID 113) and with peptides containing the scrambled sequence 'GLSQNRDVRENQRARE'. (SEQ ID 205) Synthetic peptides, which differed from the repeat motif in that single amino acids had been replaced by alanine, were probed for fibrinogen binding. Beside the spot membrane, the sequence of each synthetic peptide is listed. Bold and underlined letters indicate amino acid substitutions within the repeat motif.

Fig. 12 shows a spot membrane analysis of the fibrinogen binding repeat unit. Synthetic peptides were tested for fibrinogen binding, in which each of the amino acids of the fibrinogen binding repeat was replaced by each of the 20 amino acids. The vertical letters, printed in bold, represent the FbsA-derived fibrinogen binding sequence 'GNVLERRQRDAENRSQ'. The horizontal letters represent those amino acids that were introduced in the synthetic peptides instead of the original amino acid in the respective position.

Fig. 13 shows the competitive inhibition of fibrinogen binding to GBS 6313 by synthetic peptides. The binding assay was performed with ^{125}I-labelled fibrinogen in the presence of different concentrations of the peptides pep_FbsA (SEQ ID 211), carrying an FbsA-derived

repeat unit, and pep_R6A, possessing an R6A substitution within the repeat unit. Each experiment was performed at least in triplicate.

Fig. 14 shows eukaryotic cell adherence (A) and invasion (B) of GBS strains 6313, 706 S2, and O90R and their respective *fbsA* deletion mutants. The values represent the result of at least four independent experiments performed in triplicate. Error bars are indicated.

Fig. 15. shows a peptide ELISA of FbsA peptides with human sera. The 5 biotinylated peptides (wild type <1>: GNVLERRQRDAENRSQ SEQ ID No. 113; alanine mutant peptides: <2> GAVLERRQRDAENRSQ SEQ ID No. 207, <3> GNALERRQRDAENRSQ SEQ ID No. 208, <4> GNVLEARQRDAENRSQ SEQ ID No. 211, <5> GNVLERAQRDAENRSQ SEQ ID No. 212; see also Fig.11) were coated on Streptavidin-coated ELISA plates and analysed using 5 sera from patients infected with GBS. The patient sera were applied in a dilution of 1:200 and 1:1,000. IgG (A) and IgA (B) antibodies were detected with secondary anti-human antibodies coupled to Horse Radish Peroxidase and ABTS as substrate.

Fig. 16 shows the DNA sequence of the *pabA*/B-encoding region and the deduced PabA (nt 319-2964) and PabB (nt3087-5111) proteins from GBS 6313. Putative ribosomal binding sites (RBS) are underlined. Letters in bold and italics indicate the putative signal peptides of the deduced PabA and PabB proteins and letters in bold and underlined mark the region with high identity to the cell wall anchor motif from Gram positive bacteria.

Fig. 17 shows the DNA sequence of the *pabC*/D-encoding region and the deduced PabC (nt 487-2394) and PabD (nt 2461-3006) proteins from GBS 6313. Putative ribosomal binding sites (RBS) are underlined. Letters in bold and italics indicate the putative signal peptides of the deduced PabC and PabD proteins.

Fig. 18 shows a scanning electron microscopy of A549 cells incubated for two hours with latex beads coated with PabA, PabB, PabC, PabD, respectively. BSA-coated latex beads were used as a control.

Fig. 19 shows the adherence of GBS 6313 to and invasion of A549 cells in the presence of 100μg/ml of PabA, PabB, PabC or PabD fusion proteins. The adherence of GBS 6313 to

A549 cells (A) and its internalization into these cells (B) was arbitrarily set to 100% and the results obtained in the presence of the different fusion proteins was related to these values. Each experiment was performed at least three times in triplicate.

Fig. 20 shows eukaryotic cell adherence and internalization by GBS 6313 and its *pabA* and *pabB* deletion mutants. The adherence of GBS 6313 to A549 cells (A) and its internalization into these cells (B) was arbitrarily set to 100% and the results obtained with the GBS mutants 6313Δ*pabA* and 6313Δ*pabB* were related to these values. Each experiment was performed at least three times in triplicate.

Fig. 21 shows the testing of anti-PabA, anti-PabB, and anti-PabD antisera for their sensitivity in detecting their respective antigens. Serial dilutions of the fusion proteins PabA, PabB, and PabD were spotted onto nitrocellulose and probed with a 1:1000 dilution of the mice sera against the respective proteins. Bound antibodies were labelled with an anti-mouse-HRP conjugate and visualized by chemiluminescence.

EXAMPLES

Example 1: Experimental procedures

It is to be noted that the following materials and methods were used throughout the examples described herein if not indicated to the contrary.

Bacterial strains and culture conditions

GBS strains 6313 (serotype III) and SS1169 (serotype V) represent reference strains and have been described previously (Wibawan and Lammler, 1992). GBS strains 706 S2 (serotype Ia), 33H1A (serotype Ib), and 176 H4A (serotype II) were kindly provided by G. S. Chhatwal (GBF Braunschweig). GBS strain O90R (ATCC 12386) is a derivative of the serotype Ia strain O90. All GBS strains belonging to the serological groups Ia, Ib, II, III, and V, respectively, are clinical isolates and were isolated from infected neonates, while GBS strains from group IV were isolated from cows with mastitis (Chhatwal et al., 1984). *E. coli* DH5α (Hanahan, 1985) was used for cloning purposes and *E. coli* BL21 (Dubendorff and Studier,

1991) served as host for the production of FbsA fusion proteins. The alkaline-phosphatase-negative *E. coli* strain CC118 (Manoil and Beckwith, 1985) served as host for pHRM104-derivates and for the screening for signal-peptide encoding sequences from GBS.

GBS was cultivated at 37°C in Todd-Hewitt yeast broth (THY) containing 1% yeast extract. *E. coli* was grown at 37°C in Luria broth (LB) and clones carrying cosmid pTEX5236 or plasmid pET28a or pHRM104 were selected in the presence of chloramphenicol (15 µg/ml), kanamycin (50 µg/ml) or erythromycin (300 µg/ml). Screening for alkaline phosphatase secreting *E. coli* CC118 clones was performed on LB-plates containing 80 µ/ml X-phosphate (Sigma).

Antibodies, enzymes, peptides and human proteins

Affinity-purified rabbit anti-fibrinogen and peroxidase-labelled anti-rabbit antibodies were obtained from Dako-Biochemicals. Peroxidase-labelled goat anti-mouse antibodies were purchased from Dianova. Monoclonal anti-his-tag antibodies were obtained from Roche Diagnostics. Purified rabbit anti-fibronectin antibodies, trypsin, pronase, vitronectin, laminin, IgG, fibronectin, and fibrinogen were purchased from Sigma-Aldrich. Fibrinogen (Sigma) was passed through a gelatin-Sepharose column to remove residual contaminating fibronectin in the preparation. The purity of the fibrinogen preparation was confirmed by SDS-PAGE and Coomassie-staining and by Western blotting using anti-fibronectin antibodies. Synthetic peptides for spot membrane analysis and for inhibition experiments were synthesized as described previously (Frank and Overwin, 1996).

Plasmids and cosmids used for cloning purposes

A cosmid gene library from GBS 6313 (Reinscheid *et al.*, 2001) was used for the isolation of the *fbsA*-gene from GBS. Low-copy cosmid pTEX5236 was also used for subcloning of the *fbsA* gene after partial digestion of an *fbsA*-carrying cosmid with *Sau*3A. Plasmid pET28a (Novagen) was used for the synthesis of the hexahistidyl-tagged FbsA, PabA, PabB, PabC, and PabD fusion proteins, which were constructed as follows: A truncated *fbsA* gene, devoid of the coding region of the signal peptide and the membrane spanning domain, was PCR amplified from chromosomal DNA of GBS 6313 using the primers 1 5'GTCCTGTATCTG<u>CCATGG</u>ATAGTGTTGG (SEQ ID No. 223) and 2 5'CCGC<u>GGATCC</u>ACATTTTGATCATCACCTG (SEQ ID No. 224). The repeat-encoding region of *fbsA* was amplified with the primers 3

5′GTCCTGTATCTGCCATGGATAGTGTTGG (SEQ ID No. 225) and 4 5′CCGCGGATCCCCTATAAGTTGACCTAC (SEQ ID No. 226). Amplification of the non-repeat region of *fbsA* was performed with the primers 5 5′TGCTTTGCCATGGTAGGTCAACTTATAGGG (SEQ ID No. 227) and 6 5′CCGCGGATCCACATTTTGATCATCACCTG (SEQ ID No. 228). The *Nco*I and *Bam*HI restriction sites used for cloning are underlined. Amplification of the pabA, pabB, pabC and pabD genes, devoid of the coding region of the signal peptide and, if present, of the membrane spanning domain, was performed with the primers pabA1 5′GTGCCTTGCCATGGAAAGTACCGTACCGG (SEQ ID No. 229), pabA2 5′ GCGGACAGCTCGAGTTTCCCACCTGTCATCGG (SEQ ID No. 230), pabB1 5′GTGCCTTGCCATGGACGACGTAACAACTGATAC (SEQ ID No. 231), pabB2 5′GCGGACAGCTCGAGTGTACCAATACCACCTG (SEQ ID No. 232), pabC1 5′GTGCCTTGCCATGGGCCGGGATAACTAAAG (SEQ ID No. 233), pabC2 5′GCGGACAGCTCGAGCTCTTTTATACGCCATGAG (SEQ ID No. 234), pabD1 5′CCGCGGATCCGATGATAACTTTGAAATGCC (SEQ ID No. 235) and pabD2 5′TGGCACAAGCTTACATTCTGAGCAGAAAGC (SEQ ID No. 236).

The *Nco*I, *Xho*I and the *Bam*HI, *Hin*dIII restriction sites used for cloning are underlined. The PCR products and plasmid pET28a were digested with the indicated restriction enzymes, ligated and transformed into *E. coli* BL21. Plasmid pETfbsA-9, carrying *fbsA* with nine internal repeats, was constructed by partial digestion of pETfbsA-19 with *Xba*I, subsequent religation and transformation into *E. coli* BL21.

A plasmid library of GBS chromosomal fragmens was constructed in plasmid pHRM104 essentially as described elsewhere (Pearce *et al.*, 1993). Briefly, chromosomal DNA from GBS 6313 was fractionated by sonication for 45 sec, the obtained fragments were blunt-ended by Klenow polymerase, ligated into SmaI digested pHRM104, and the ligation mixture transformed into *E. coli* CC118. Transformants were plated onto erythromycin and X-phosphate containing agar plates and incubated for three days.

Southern and blot analysis

Chromosomal DNA from GBS was prepared as described elsewhere (Pospiech, 1995). Digoxigenin-labelled probes of the inserts in plasmid pHRM104 were obtained by PCR with

the primers 7 5′AATATCGCCCTGAGC (SEQ ID No. 237) and 8 5′GGTTTTCCCAGTCACG (SEQ ID No. 238). The same primers were also used for sequencing the inserts in the pHRM104 derivates. Digoxigenin-labelled probes of the genes *fbsA*, *pabA/B* and *pabC/D*, respectively, were obtained by PCR with the primers *fbsA1* 5′GTCCTGTATCTGCTATGGATAGTGTTGG (SEQ ID No. 239), *fbsA2* 5′ACATTTTGATCATCACCTG (SEQ ID No. 240), *pabA* 5′ACTGCTGAGCTAACAGGTG (SEQ ID No. 241), *pabB* 5′ ACATCACCTGACAATGTCGC (SEQ ID No. 242), *pabC* 5′GCGATTGTGAATAGAATGAG (SEQ ID No. 243), and *pabD* 5′TATACAAAGCCTGAGCTTC (SEQ ID No. 244). To analyze the distribution of the genes *fbsA*, *pabA/B* and *pabC/D* among different clinical isolates of GBS, their chromosomal DNA was digested with *Hind*III, *Bst*EII or *Nco*I and hybridized to the *fbsA*-, *pabA/B*- or *pabB/C* specific probe. Labelling, hybridization, washing and detection in Southern blots was performed using the Dig-labelling and detection kit (Roche Diagnostics) according to the instructions of the manufacturer with subsequent detection by chemiluminescence.

PCR-amplification and sequencing of fbsA from different GBS strains

The *fbsA* gene was amplified from the chromosome of the GBS strains 706 S2, 33H1A, 176 H4A, O90R and SS1169 by PCR using the primers 9 5′TTACCGTAGCCTGTATCACC (SEQ ID No. 245) and 10 5′CGACCTACGATAGCAACG (SEQ ID No. 246) and the PCR products were subsequently sequenced. The nucleotide sequence of the *fbsA* gene from strain 6313 was obtained by sequencing the 2.6 kb insert of pTEXfbsA.

Construction of fbsA deletion mutants

The thermosensitive plasmid pG$^+$host6 (Appligene) was used for targeted deletion of the *fbsA* gene in the GBS strains 6313, 706 S2, and O90R, respectively . Two fragments flanking the *fbsA* gene were amplified by PCR from chromosomal DNA of GBS 6313 using the primer pairs *fbsA*_del1 5′CCGC<u>GGATCC</u>GAATATGCTACCATCAC (SEQ ID No. 247) and *fbsA*_del2 5′*CCCATCCACTAAACTTAAACA*TTCCTGATTTCCAAGTTC (SEQ ID No. 248) as well as *fbsA*_del3 5′*TGTTTAAGTTTAGTGGATGGG*GCTGCGGTTTGAGACGC (SEQ ID No. 249) and *fbsA*_del4 5′TGGCAC<u>AAGCTT</u>TACCTGCTGAGCGACTTG (SEQ ID No. 250). Complementary DNA sequences in the primers *fbsA*_del2 and *fbsA*_del3 are marked in italics and the *Bam*HI and *Hind*III restriction sites in the primers *fbsA*_del1 and *fbsA*_del4 are underlined. The *fbsA* flanking PCR products were mixed in equal amounts with

each other and subjected to crossover PCR by using primers *fbsA*_del1 and *fbsA*_del4. The resulting PCR product consisted of the *fbsA* flanking regions on a single DNA fragment. The crossover PCR product and plasmid pG^{+}host6 were digested with *Bam*HI and *Hin*dIII, ligated and transformed into *E. coli* DH5α. The resulting plasmid, pG^{+}Δ*fbsA* was transformed into the GBS strains 6313, 706 S2, and O90R, respectively, and transformants were selected by growth on erythromycin agar at 30°C. Cells in which pG^{+}Δ*fbsA* had integrated into the chromosome were selected by growth of the transformants at 39°C with erythromycin selection as described (Maguin *et al.*, 1996). Four of such integrants from each strain were serially passaged for three days in liquid medium at 30°C without erythromycin selection to facilitate the excision of plasmid pG^{+}Δ*fbsA*, leaving the desired *fbsA* deletion in the chromosome. Dilutions of the serially passaged cultures were plated onto agar and single colonies were tested for erythromycin sensitivity to identify pG^{+}Δ*fbsA* excisants. Chromosomal DNA of the parental GBS strains 6313, 706 S2, and O90R, respectively, and of 10 erythromycin sensitive GBS excisants from each strain was tested by Southern blot after *Hin*dIII digestion using a digoxigenin-labelled *fbsA* flanking fragment obtained with the primers *fbsA*_del3 and *fbsA*_del4.

Construction of pabA and pabB deletion mutants

Deletion mutants in the genes pabA and pabB, respectively, were constructed in GBS 6313 as described for the construction of fbsA deletion mutants. The primer pairs used to construct the pabA deletion mutant were *pabA*_del1 5′GTTAAAGGTAACCTGCCTG (SEQ ID No. 251), *pabA*_del2 5′*CCCATCCACTAAACTTAAACA*TACAACTCCTATTGTGCCGAAATGTCG (SEQ ID No. 252) as well as *pabA*_del3 5′*TGTTTAAGTTTAGTGGATGGG*CACTTAGAGATTTTCCAATCC (SEQ ID No. 253) and *pabA*_del4 5′GACATCATAGATCCACC (SEQ ID No. 254). After cross-over PCR the resulting PCR fragment and vector pG^{+}host6 were digested with *Hin*dIII and *Eco*RI and subsequently ligated, resulting in plasmid pG^{+}Δ*pabA*. The primer pairs for deleting *pabB* were *pabB*_del1 5′CCGC*GGATCC*GGAGCTACGTTTGAACTTC (SEQ ID No. 255), *pabB*_del2 5′*CCCATCCACTAAACTTAAACA*ATATTACCGCAGCACCAC (SEQ ID No. 256) as well as *pabB*_del3 5′*TGTTTAAGTTTAGTGGATGGG*ACAAGAAGGCCAAGAAGG (SEQ ID No. 257) and pabB_del4 5′CACGCAACGC<u>GTCGAC</u>GCACAGCTTTAACTGTAC (SEQ ID No. 258). The *Bam*HI and *Sal*I restriction sites are underlined. The fragment obtained by cross-over PCR and the vector pG^{+}host6 were digested with *Bam*H1 and *Sal*I and ligated, resulting in plasmid

pG$^{+}\Delta$*pabB*. Plasmids pG$^{+}\Delta$*pabA* and pG$^{+}\Delta$*pabB* were subsequently transformed into GBS 6313. The procedure for the generation of *pabA* and *pabB* deletion mutants was identical to that for the constrution of *fbsA* deletion mutants.

General DNA techniques

Conventional techniques for DNA manipulation, such as restriction enzyme digests, PCR, ligation, transformation by electroporation and Southern blotting were performed as described by Sambrook *et al.* (Sambrook *et al.*, 1989).

Binding of soluble ^{125}I-labelled fibrinogen to GBS

Purified human fibrinogen was radiolabelled with ^{125}I, using the chloramin T method (Hunter and Greenwood, 1962). Binding of labelled fibrinogen to GBS was performed essentially as described by Chhatwal *et al.* (1983). Briefly, overnight cultures of GBS were pelleted by centrifugation, washed twice with phosphate-buffered saline supplemented with 0.02% tween 20 (PBST) and adjusted photometrically to a transmission of 10% at 600 nm. A total of 0.2 ml of the bacterial suspension was added to 20 µl of ^{125}I-labelled fibrinogen containing 23 ng of fibrinogen. After incubation for 1 h at room temperature, the streptococci were sedimented by centrifugation and washed with 1 ml of PBST. The radioactivity of the pellet was finally measured in a gamma counter (Packard Instruments). The amount of bacterial-bound fibrinogen was calculated as the percentage of total radiolabelled fibrinogen added to the bacteria. In inhibition experiments, the binding of 23 ng of radiolabelled fibrinogen to 0.2 ml of GBS (T=10%) was determined in the presence of various amounts of FbsA fusion proteins, Bsp fusion protein or synthetic peptides. Each experiment was repeated at least three times in triplicate.

Binding of FITC-labelled GBS to immobilized fibrinogen

Terasaki plates were coated with human fibrinogen and the binding of FITC-labelled bacteria to the immobilized fibrinogen was measured as described by Podbielski *et al.* (Podbielski *et al.*, 1999). In brief, 10µl of a 100µg/ml stock solution of human fibronectin, fibrinogen, laminin and collagen I and IV, respectively, was added to each well and incubated overnight at room temperature in a moist chamber. Subsequently, the microtiter plates were washed with PBS and residual buffer was carefully removed. FITC-labelling of GBS was performed with cultures in the exponential (OD_{600}: 0.5) and in the stationary (OD_{600}: 1.5) growth phase. 12 ml of bacterial culture were pelleted by centrifugation, washed with 12 ml of PBS and

resuspended in 2 ml FITC-solution (1 mg/ml FITC in 50 mM sodium carbonate buffer, pH 9.2). Following a 20 min incubation in the dark, the cells were pelleted by centrifugation, washed twice with PBS and sonicated for 20 sec to disrupt bacterial chains. The bacterial suspension was adjusted to an OD_{600}:1.0 with PBS, vortexed vigurously and kept in the dark until use. 10 µl of FITC-labelled GBS suspension was addet to each Terasaki well coated with different human proteins. After a 60 min incubation at 37°C, unbound bacteria were removed by five washes with PBS and bound bacteria were fixed with 0.5% glutaraledhyde for 5 min. The plates were finally washed twice with PBS and the fluorescence of each well was determined in an automated Cyto Fluor II fluorescence reader (PerSeptive Biosystems) at excitation and detection wavelengths of 485 nm and 530 nm, respectively. The efficiency of FITC-labelling of the bacteria was determined by incubating 500 µl of the FITC-labelled bacteria for 60 min at 37°C, three washes of the bacteria with PBS, resuspension of the cells in 500 µl of PBS and measuring the fluorescence of 10 µl aliquots of the suspension in uncoated Terasaki mitrotiter plates. Each assay was measured in triplicate and repeated at least four times.

Preparation and purification of fusion proteins

The different FbsA fusion proteins as well as the fusion proteins PabA, PabB, PabC, PabD, and Bsp (Reinscheid *et al.*, 2002) were synthesized in recombinant *E. coli* BL21 by the addition of 1 mM IPTG after the culture had reached an optical density of 1.0. The cells were disrupted using a French Press cell and purification of the fusion protein was performed according to the instructions of Qiagen using Ni^{2+} affinity chromatography. Subsequently, the PabA, PabB and PabC fusion proteins were dialyzed against 20 mM Tris/HCl, pH 8.5 and loaded onto a MonoQ anion exchange column (Amersham/Pharmacia). A linear gradient from 0 M to 1.0 M NaCl in 20 mM Tris/HCl was used to elute the fusion proteins from the column. For further purification of PabD, the fusion protein was dialyzed against 20 mM Tris/HCl buffer and loaded onto a MonoS cation exchange column (Amersham/Pharmacia). A linear gradient from 0 M to 1.0 M NaCl in 20 mM Tris/HCl buffer was used for the elution of PabD. All fusion proteins were finally dialyzed against PBS and stored at –20°C.

Screening for fibrinogen-binding colonies

Cosmid-carrying *E. coli* clones were transferred in duplicate to tetracycline containing LB plates and incubated overnight. The next day the colonies of one plate were transferred to nitrocellulose for 6 h. The cells on the filter were lysed by chloroform vapour for 20 min and

subsequently incubated overnight in PBS with 1 mg/ml lysozyme and 1 mM PMSF. The membrane was blocked overnight with 10% skim milk in phosphate-buffered saline (PBS) and subsequently probed for binding of human fibrinogen as described below.

Western Blot and spot membrane analysis

In Western blot experiments proteins were separated by SDS-PAGE and electroblotted onto nitrocellulose. The membrane was subsequently blocked overnight with 10% skim milk in PBS. For spot membrane experiments peptides of 16 amino acids were synthesized and equal amounts of the peptides were directly spotted onto cellulose paper as described previously (Frank and Overwin, 1996). Blocking was performed in membrane blocking solution (MBS) that consisted of 20 ml casein based blocking buffer (Genosys Biotechnologies, Cambridge, England), 80 ml Tris-buffered saline (TBS), 0.05% tween 20, and 5 g sucrose. Probing for fibrinogen-binding was performed as described below.

Detection of fibrinogen binding by Western blot, spot membrane and colony blot

Membranes that had been blocked overnight were incubated for 1 h with 2 μg/ml of human fibrinogen. For Western and colony blot experiments, fibrinogen and antibodies were diluted in PBS while for spot membrane analysis they were diluted in MBS. Following three washes with PBS, the membrane was incubated with anti-fibrinogen antibodies (1:1000 in PBS or MBS) for 1 h. This incubation was followed by three washes with PBS, containing 0.05% tween 20 (PBST) and two washes with PBS. Subsequently, the membrane was incubated for 1 h with peroxidase-labelled anti-rabbit IgG (1:1000 in PBS or MBS). After three washes with PBST and two washes with PBS, bound fibrinogen was detected by chemiluminescence using the ECL-kit (Amersham/Pharmacia). In control experiments, no cross-reactivity of the used antibodies with the immobilized proteins and peptides was detected.

Opsonophagocytosis assay

Resistance to phagocytosis was measured as described by Podbielski *et al.* (1996). Briefly, a growing culture of GBS was adjusted to 10^3 colony forming units per millilitre. 100 μl of the suspension were added to 300 μl of heparinized human blood and the reaction mixture was incubated at 37°C with end-over-end rotation for 3 h. Pre- and postincubation aliquots were serially diluted and plated onto THY agar for overnight culture. For each strain the ratio of colony-forming units prior to, and following 3 h incubation with human blood was calculated. Each experiment was performed three times in triplicate.

Epithelial cell adherence and internalization assay

Adherence of GBS to epithelial cells and internalization into epithelial cells was assayed essentially as described previously (Caparon *et al.*, 1991; Rubens *et al.*, 1992). Briefly, A549 cells were transferred to 24-well tissue culture plates at approximately 4 x 10^5 cells per well and cultivated overnight in RPMI (Gibco BRL) tissue culture medium, supplemented with 10% of fetal calf serum. After replacement of the medium with 1 ml of fresh medium, the cells were infected with $5x10^6$ streptococci per well and incubated at 37°C for 2 h. The non-adherent bacteria were removed by washing three times with PBS. In adherence assays, the epithelial cells were subsequently detached from the well by the addition of trypsin/EDTA and lysed by adding 300 µl of distilled water. Adherent bacteria were quantitated by plating serial dilutions of the lysate onto THY agar plates. For internalization assays the epithelial cells were incubated after 2 h of infection for another 2 h in tissue culture medium supplemented with penicilling G (10 U) and streptomycin (0.01 mg) to kill extracellular bacteria. After three washes with PBS, the epithelial cells were detached by the addition of trypsin/EDTA and lysed in 300 µl of distilled water. The amount of intracellular bacteria was quantified by plating serial dilutions of the lysate onto THY agar plates. Each experiment was repeated at least three times in triplicate.

In competition studies, 1 ml of fresh tissue culture medium containing 50 µg of purified fusion protein or 1 µg of fibrinogen was added to the A549 cells and subsequently, the cells were infected with GBS 6313.

Interaction of protein-coated latex beads with A549 cells

Approximately 108 latex beads (3 µm diameter, Sigma) were washed tree times in PBS and then coated with 300 µg of fusion protein or BSA in 500 µl PBS overnight at 4°C. Coated beads were washed once in PBS and then blocked with 200 µl of 10 mg/ml BSA in PBS for 1 h at room temperature. Beads were washed twice in PBS and once in RPMI + 10% FCS and then resupended in 1 ml of RPMI + 10% FCS. 300 µl of beads were added to approximately 4 x 10^5 A549 cells in 24-well plates. The cells were incubated for 1 h at 37°C (5% CO_2), washed five times with PBS and fixed in a solution containing 3% glutaraldehyde and 5% formaldehyde in cacodylate bluffer for 45 min on ice. The samples were washed with cacodylate buffer, dehydrated in a graded series of acetone and subjected to critical point

drying with CO_2. Samples were then coated with a 10 nm thick gold film and examined by scanning electron microscopy as described previously (Reinscheid *et al.*, 2001).

Synthesis of biotinylated peptides

Peptides were synthesized in small scale (4 mg resin; up to 288 in parallel) using standard F-moc chemistry on a Rink amide resin (PepChem, Tübingen, Germany) using a SyroII synthesizer (Multisyntech, Witten, Germany). After the sequence was assembled, peptides were elongated with Fmoc-epsilon-aminohexanoic acid (as a linker) and biotin (Sigma, St. Louis, MO; activated like a normal amino acid). Peptides were cleaved off the resin with 93%TFA, 5% triethylsilane, and 2% water for one hour. Peptides were dried under vacuum and freeze dried three times from acetonitrile/water (1:1). The presence of the correct mass was verified by mass spectrometry on a Reflex III MALDI-TOF (Bruker, Bremen Germany). The peptides were used without further purification.

Enzyme linked immune assay (ELISA).

Biotin-labeled peptides were coating on Streptavidin ELISA plates (EXICON) at 10 µg/ml concentration according to the manufacturer's instructions. Sera were tested at two dilutions, 200X and 1,000X.

Highly specific Horse Radish Peroxidase (HRP)-conjugated anti-human IgG or anti-human IgA secondary antibodies (Southern Biotech) were used according to the manufacturers' recommendations (dilution: 1,000x). Antigen-antibody complexes were quantified by measuring the conversion of the substrate (ABTS) to colored product based on OD_{405nm} readings in an automated ELISA reader (TECAN SUNRISE). Following manual coating, peptide plates were processed and analyzed by the Gemini 160 ELISA robot (TECAN) with a built-in reader (GENIOS, TECAN).

Example 2: Identification of a novel *S. agalactiae* adhesion by a signal peptide tagging screen.

Results

GBS strain 6313, belonging to serotype III, was tested in binding experiments for its

interaction with radiolabelled human vitronectin, laminin, fibronectin, fibrinogen, and IgG. Strain 6313 accumulated about 50% of the total fibrinogen on its surface. Of the other proteins tested, none interacted in significant amounts (> 5%) with GBS 6313. Treatment of the bacteria with either trypsin or pronase reduced the amount of bound fibrinogen to levels below 5%, indicating a proteinacious nature of the fibrinogen-binding structures of GBS 6313.

An *Escherichia coli* cosmid gene library of GBS 6313 was screened by colony blotting for the presence of fibrinogen-binding *E. coli* clones, resulting in the identification of a clone that revealed strong interaction with human fibrinogen. Partial digestion of its cosmid with *Sau*3A and subcloning of fragments in the range of 2-3 kb in plasmid pTEX5236 resulted in the isolation of plasmid pTEXfbsA, carrying a 2.6 kb insert that conferred fibrinogen-binding to *E. coli* DH5α. The insert of pTEXfbsA was sequenced and the analysis of the obtained sequence identified one open reading frame of 1329 bp, designated *fbsA* as it encodes a fibrinogen-binding protein from *S. agalactiae* (Fig. 1). The *fbsA* gene is preceded by a typical ribosomal binding site (AGGAGA) and followed by a sequence resembling a transcriptional terminator ($\Delta G°$=-18 kcal/mol). Analysis of the *fbsA*-encoding region revealed for the deduced FbsA protein typical features of a surface-located protein from streptococci (Fig. 1), i.e. a signal peptide sequence of 35 amino acids (Nielsen *et al.*, 1997) at its N-terminus and a cell wall anchor motif (LPKTG) (Schneewind *et al.*, 1993) at its C-terminus. The *fbsA* gene encodes a primary translation product of 442 amino acids (Mr 51319) which is putatively processed posttranslationally to yield a mature protein of 378 amino acids (Mr 44260). The most striking feature of FbsA is its highly repetitive nature: FbsA carries 19 complete repeats of 16 amino acids that are almost identical. 14 of the 19 repeats are comprised of the sequence motif 'GNVLERRQRDAENRSQ' while two repeats (3 and 10) carry an R14K substitution and three repeats (2, 9, and 19) possess both an A11V and an R14K substitution.

Southern blot experiments with clinical GBS isolates, belonging to the serotypes Ia, Ib, II, III, IV, and V, were performed to analyze the presence of *fbsA* in GBS. By Southern blot analysis, the *fbsA* gene was detected in 25 of 27 strains (Fig. 2), indicating a wide distribution of *fbsA* in different serotypes of GBS. Interestingly, the size of the *fbsA* gene varied significantly between the individual strains in the Southern blot analysis. To unravel the molecular basis of this size variation, the *fbsA* gene was amplified by PCR from the GBS strains 706 S2 (serotype Ia), 33H1A (serotype Ib), O176 H4A (serotype II), SS1169 (serotype V), and O90R

(a capsule mutant derived from a serotype Ia strain) and sequenced. Analysis of the obtained sequences revealed one open reading frame in each PCR product with high identity to *fbsA* from GBS strain 6313 (Figs. 3-7). Analysis of the deduced FbsA proteins identified in all of them a putative signal peptide at their N-termini and a putative cell wall anchor at their C-termini. As expected from the Southern blot experiments, the size of the single proteins is significantly different. The primary translation product of *fbsA* is 410 amino acids for strain 706 S2 (Fig. 3), 346 amino acids for strain 33H1A (Fig. 4), 186 amino acids for strain 176 H4A (Fig. 5), 298 amino acids for strain O90R (Fig. 6), and 618 amino acids for strain SS1169 (Fig. 7). As shown in Fig. 8, the different sizes between the single FbsA proteins are exclusively due to a different number of repeats within the individual proteins. Fig. 8 also shows, that the individual repeats of the deduced FbsA proteins reveal differences in their amino acid composition. Thus, the *fbsA* gene from different GBS strains appears to be highly variable in the number of and flexible in the composition of single repeat-encoding units.

Example 3: FbsA is the fibrinogen receptor of *Streptococcus agalactiae*.

Results

For functional analysis of FbsA, a truncated FbsA polypeptide (FbsA-19), devoid of a signal peptide and a membrane-spanning region was synthesized as a hexa-histidyl fusion protein in *E. coli* BL21 and purified by affinity chromatography. In Western blot experiments FbsA-19 revealed binding to human fibrinogen (Fig. 9), confirming FbsA as a fibrinogen receptor from GBS. To localize the fibrinogen-binding region in the FbsA protein, the N-terminal and the C-terminal regions of FbsA were synthesized as FbsA-N and FbsA-C fusion proteins and tested for fibrinogen binding. As shown in Fig. 9, fibrinogen binding was observed for FbsA-N but not for FbsA-C, indicating that the N-terminal repeats of FbsA mediates fibrinogen binding.

In competitive inhibition experiments with ^{125}I-labelled fibrinogen, different proteins were tested for their capability to interfere with the binding of radiolabelled fibrinogen to GBS. As a control, the non-fibrinogen binding surface protein Bsp from GBS (Reinscheid *et al.*, 2002) was tested for inhibiting the binding of fibrinogen to GBS. As shown in Fig. 10, the addition of increasing concentrations of Bsp had no effect on fibrinogen binding by GBS. However, increasing concentrations of purified FbsA-19 substantially inhibited the binding of ^{125}I-

labelled fibrinogen to GBS 6313 cells. To analyse, if the number of repeats of FbsA has an effect on fibrinogen binding, a derivative of FbsA with only 9 repeats (FbsA-9) was tested for its capability to inhibit fibrinogen binding by GBS. Interestingly, significantly higher concentration of FbsA-9 had to be used to obtain a comparable inhibition of fibrinogen binding as obtained with FbsA-19. This finding indicates that increasing numbers of repeats either increases the affinity of FbsA for fibrinogen and/or supports a higher amount of fibrinogen to be bound by FbsA.

To further characterize the interaction of FbsA and fibrinogen on the molecular level, FbsA-derived synthetic peptides were tested for their interaction with human fibrinogen. At first, we analysed a single repeat unit of FbsA (GNVLERRQRDAENRSQ) for its capability to interact with human fibrinogen. In Dot Blot experiments a strong interaction of this synthetic peptide with human fibrinogen was observed while a randomised peptide containing the identical amounts of amino acids but in different order, showed no binding of fibrinogen (Fig. 11). This result shows that a single repeat unit of FbsA is capable of specific binding to human fibrinogen. To identify amino acids in the repeat region that are essential for fibrinogen binding, we synthesized peptides that contained single alanine replacements at different positions. Testing of these peptides for their interaction with fibrinogen (Fig. 11) identified N^2, V^3, L^4, R^6, and R^7 of the repeat sequence to be essential for fibrinogen binding. Furthermore, substitution of G^1, R^9, and R^{14} by alanine significantly reduced the interaction of the repeat unit with human fibrinogen.

A comprehensive analysis of fibrinogen binding by the 16 amino acid sequence motif was performed to identify putative conservative substitutions within the repeat regions. Therefore, synthetic peptides, derived from the sequence motif 'GNVLERRQRDAENRSQ' were synthesized and directly spotted onto a membrane. Every peptide differed from each other by a single amino acid substitution. In this way, every amino acid within the repeat was successively replaced by one of the twenty proteinacious amino acids. Testing of the individual spots for fibrinogen binding resulted in a complex picture of the interaction between fibrinogen and the repeat unit (Fig. 12). Replacement of G^1 by any other amino acid reduced the fibrinogen binding of the repeat although binding was not completely abolished. N2S and N2T substitutions did not affect fibrinogen binding, although replacement of N^2 by any other amino acid significantly reduced fibrinogen binding. V^3 and L^4 could not be replaced by other amino acids without significant reduction of binding function. Fibrinogen

binding was not affected by E5A, E5M and E5Q substitutions but any other amino acid in this position resulted in a lower binding of fibrinogen. Substitutions of R^6 predominantly caused a loss of fibrinogen binding while peptides with R6A, R6K and R6W substitutions retained little binding activity. However, replacement of R^7 by any other amino acid resulted in a loss of fibrinogen binding. Q^8 could be substituted by many amino acids without an effect on binding while R^9 could only be replaced by K or W without affecting binding. D10A, D10E, D10N, and D10Q substitutions had no effect on fibrinogen binding while the same was true for A11F, A11I, A11L, A11V and A11Y changes. E^{12} and N^{13} could be replaced by a variety of amino acids without affecting binding. In contrast, only R14K substitutions retained fibrinogen binding of the peptide. Finally, S^{15} and Q^{16} could be replaced by many other amino acids without loss of binding function. Derived from the result of the spotting membrane experiment, the following fibrinogen binding motif can be postulated: G-N/S/T-V-L-A/E/M/Q-R-R-X-K/R/W-A/D/E/N/Q-A/F/I/L/V/Y-X-X-K/R-X-X (SEQ ID No. 222). This consensus motif could not be identified in fibrinogen binding proteins from other organisms, indicating that it represents a novel type of fibrinogen binding site.

Derived from the results of the spot membrane analysis, two different synthetic peptides were tested for their capability to inhibit fibrinogen binding of GBS. One peptide (pep_FbsA) represented the original repeat unit sequence 'GNVLERRQRDAENRSQ' (SEQ ID No. 113) while the other peptide (pep_R6A) carried an R6A substitution. In spot membrane analysis, the latter peptide had revealed a significantly reduced binding to fibrinogen. In competitive inhibition experiments, both peptides were tested for inhibiting the binding of radiolabelled fibrinogen to GBS (Fig. 13). A concentration of 160 µM of pep_FbsA inhibited fibrinogen binding by 80% whereas the same concentration of pep_R6A caused only 20% inhibition of fibrinogen binding. These findings demonstrate that the soluble form of the repeat unit of FbsA is capable of fibrinogen binding. Furthermore, the difference in the inhibition of fibrinogen binding between the two peptides confirms the results of the spot membrane analysis and shows that R^6 plays an important role in fibrinogen binding.

To analyse the contribution of FbsA for the fibrinogen binding of GBS, *fbsA* deletion mutants were constructed in the GBS strains 6313, 706 S2, and O90R, respectively. Southern blot analysis revealed the successful deletion of *fbsA* in the respective strains (data not shown), which were termed accordingly 6313Δ*fbsA*, 706 S2Δ*fbsA*, and O90RΔ*fbsA*. Mutants and parental strains were subsequently tested for their binding of soluble and immobilized

fibrinogen. While GBS strains 6313, 706 S2 and O90R exhibited about 50%, 8%, and 12% binding of ^{125}I-labelled soluble fibrinogen, their respective *fbsA* mutants bound less than 2%. Similarly, in binding experiments using FITC-labelled bacteria, about 45%, 15%, and 24% of the total bacteria from the GBS strains 6313, 706 S2, and O90R bound to immobilized fibrinogen but less than 2% of the respective *fbsA* mutants interacted with the immobilized fibrinogen. From these results it can be concluded that FbsA is the major fibrinogen-binding protein in the GBS strains 6313, 706 S2, and O90R, respectively, and that it mediates the binding of the bacteria both to soluble and to immobilized fibrinogen.

Example 4: FbsA contributes to adherence and invasion of epithelial cells and inhibits opsonophagocytosis.

Results

To analyse the importance of FbsA for protecting GBS from opsonophagocytosis, the GBS strains 6313 and 6313Δ*fbsA* were tested for survival in a classical bactericidal assay in whole human blood. After inoculation of heparinized human blood with 100±30 colony forming units (cfu) of either of the two strains, both strains revealed growth, however, after three hours of incubation, strain 6313 grew to 2500±500 cfu/assay while strain 6313Δ*fbsA* grew only to 800±100 cfu/assay. This finding indicates a role of FbsA in preventing opsonization.

The GBS strains 6313, 706 S2 and O90R, and their respective *fbsA* deletion mutants were also tested for their ability to adhere to and invade the human lung epithelial cell line A549. As shown in Fig. 14A, the adhesion of the *fbsA* deletion mutants to A549 cells was significantly impaired compared to their parental strains. Similarly, the ability of the *fbsA* deletion mutants to invade A549 cells was also drastically reduced (Fig. 14B). To analyse this effect in more detail, the ability of GBS 6313 to adhere to and to invade A549 cells in the presence of 1μg/ml of externally added fibrinogen was quantitated. The addition of fibrinogen resulted in a 90% reduction of the adherence of GBS 6313 to and invasion of A549 cells. Taken together, these findings indicate that in GBS the binding of FbsA to fibrinogen plays an important role in the bacterial adhesion to and invasion of human epithelial cells.

Example 5: FbsA is highly immunogenic in humans.

Results

Five sera from patients were analysed for the presence of antibodies directed against 5 peptides (wild type <1>: GNVLERRQRDAENRSQ (SEQ ID No. 113); alanine mutant peptides: <2> GAVLERRQRDAENRSQ (SEQ ID No. 207), <3> GNALERRQRDAENRSQ (SEQ ID No. 209), <4> GNVLEARQRDAENRSQ (SEQ ID No. 211), <5> GNVLERAQRDAENRSQ (SEQ ID No. 212); see Fig.11). Besides the wild type sequence of the repeat region, 4 peptides with alanine substitutions were chosen, devoid of fibrinogen binding activity. The elimination of fibrinogen binding activity of the peptides was sought in order to evaluate whether fibrinogen may interfere with the binding antibodies. All peptides were synthesized with a N-terminal biotin-tag and used as coating reagents on Streptavidin-coated ELISA plates.

The ELISA analysis was performed with the Gemini 160 ELISA robot. IgA and IgG antibody levels are presented for the indicated sera with all five peptides (Fig.15). Of the five sera chosen for this analysis mainly one showed a very high reactivity with the analysed peptides. Comparing the wild type and mutant peptides, the mutant peptides 2, 3 and 4 showed similar reactivities with both IgA and IgG antibodies, whereas the wild type peptide and peptide 5 were less well recognized by all sera. For the wild type peptide, this is probably explained by the presence of fibrinogen in human serum, which may compete with antibody binding to the peptide. The mutation in peptide 5 may have changed binding of the antibodies and therefore reduced reactivity. Interestingly, the reactivities of the peptides were very high with IgA antibodies and less pronounced with IgG, indicating that the antibody response in humans mainly involves the production of IgA antibodies, which are especially important for the prevention of colonization. These data are a strong indication that the FbsA protein is expressed in vivo during infection and that it is surface accessible for human antibodies.

Example 6: Identification of additional *S. agalactiae* adhesions by the signal peptide tagging screen.

Results

For the identification of further adhesins and invasins from GBS, chromosomal DNA from GBS 6313 was fragmented by sonication, the obtained fragments were filled in by Klenow polymerase treatment, subsequently ligated into plasmid pHRM104 and transformed in *E. coli* CC118. After screening on X-phosphate containing LB-plates, four colonies were surrounded by a wide blue halo. The plasmids of these clones were isolated and their inserts were sequenced. Analysis of the obtained sequences identified four incomplete open reading frames, each starting with a signal-peptide-encoding sequence. As the genes represented potential adhesins from group B streptococcus, they were named *pabA*, *pabB*, *pabC*, and *pabD*, respectively. Digoxigenin-labelled probes were amplified from the four incomplete genes by PCR. The DNA probes were used for screening a GBS 6313 cosmid gene bank in *E. coli*, resulting in the identification of one *E. coli* clone that hybridised with both the *pabA* and *pabB* probe and one *E. coli* clone that revealed hybridisation with both the *pabC* and *pabD* probe. From these clones cosmid DNA was isolated and the complete sequence of the genes *pabA-D* was determined by sequencing. Analysis of the obtained sequence information revealed that the *pabA* gene is located in front of the *pabB* gene (Fig. 16), while the *pabC* gene is preceding the *pabD* gene (Fig. 17). The genes *pabA*, *pabB*, *pabC*, and *pabD* encode proteins of 901 aa, 674 aa, 643 aa, and 182 aa, respectively. By the method of Nielsen *et al.* (1997), a putative signal peptide of 32 aa, 29 aa, 26 aa, and 23 aa could be predicted for the proteins PabA, PabB, PabC and PabD, respectively (Figs 16 and 17). In addition, the proteins PabA and PabB carry at their C-terminus the sequences IPMTG and IPQTG, respectively, which reveal high identity to cell wall anchor motifs of Gram-positive bacteria. By Southern Blot analysis, the genes *pabA-D* were detected in 90-95% of 35 tested clinical GBS isolates, indicating a wide distribution of these genes in GBS.

Example 7: PapA-D contribute to adhesion and invasion of GBS to human epithelial cells.

Results

To analyse the importance of the four proteins for the adhesion of GBS to epithelial cells, the genes *pabA* and *pabB* were cloned devoid of their signal peptide encoding sequence and cell wall anchor motif in the *E. coli* expression vector pET28a, placing a hexa-histidyl tag at the C-terminus of the PabA and PabB fusion proteins. In parallel, the genes *pabC* and *pabD* were cloned devoid of their signal peptide encoding sequence in pET28a, resulting in the synthesis of the C-terminally his-tagged fusion proteins PabC and PabD. After construction of the plasmids in *E. coli* DH5α, the constructs were transformed in *E. coli* BL21 (DE) and the synthesis of the fusion proteins was induced by the addition of IPTG. The different fusion proteins were subsequently purified by Ni^{2+}-affinity chromatography. The proteins PabA, PabB, and PabC were further purified by cation exchange chromatography and the PabD protein was purified to homogeneity by anion exchange chromatography. The purified proteins were coated onto latex beads and the beads were allowed to interact with the human lung epithelial cell line A549. As a control, bovine serum albumin (BSA) coated beads were also allowed to bind to A549 cells. As shown in Fig. 18, BSA coated beads revealed no interaction with lung epithelial cells while beads coated with the proteins PabA, PabB, PabC or PabD revealed significant binding to A549 cells. This finding indicates that the proteins PabA, PabB, PabC and PabD mediate bacterial binding to host cells. In competition experiments, the adhesion of GBS 6313 to A549 cells and the invasion of the bacteria into this cell line were quantitated in the absence and in the presence of purified PabA, PabB, PabC or PabD fusion protein. As shown in Fig. 19, the addition of PabA, PabC and PabD significantly reduced the ability of GBS 6313 to adhere to and to invade A549 cells. Surprisingly, the addition of PabB increased the adhesion of GBS 6313 to and the invasion of A549 cells. This observation again supports the idea of PabA, PabB, PabC and PabD being adhesins of GBS.

To analyse this effect further, the genes *pabA* and *pabB*, respectively, were deleted in the chromosome of GBS 6313. The resultant mutants were tested for their adhesion to and invasion of epithelial cells. Compared to the parental GBS strain 6313, both mutants revealed an about 50% reduction in their adherence to and invasion of A549 cells (Fig. 20).

Taken together, these data suggest, that the proteins PabA, PabB, PabC and PabD, respectively, play a role in the adhesion of GBS to and the invasion of epithelial cells.

To test, if the proteins PabA, PabB and PabD elicit an immune response in mice, purified PabA, PabB and PabD fusion protein was used for the subcutaneous immunization of mice. The mice were boosted after three weeks and serum was collected six weeks after the first immunization. Serial dilutions of the PabA, PabB, and PabD fusion proteins were blotted onto nitrocellulose and probed with the mice sera against the different proteins. As depicted in Fig. 21, the fusion proteins PabA, PabB and PabD were sensitively detected by their respective antisera, indicating a high immunogenicity of the three proteins in mice.

The following is a list of all of the papers and documents referred to herein. It is to be understood that the whole disclosure of these references is hereby incoropráted herein by reference.

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The features of the present invention disclosed in the specification, the claims and/or the drawings may both separately and in any combination thereof be material for realizing the invention in various forms thereof.

InterCell AG
I 10002 EP

Nucleic acids coding for adhesion factors of group B streptococcus, adhesion factors of group B streptococcus and uses thereof

Claims

1. An isolated nucleic acid molecule which is selected from the group comprising
   a) a nucleic acid having at least 70% identity to a nucleic acid sequence which is selected from the group comprising SEQ ID NO 1 to SEQ ID NO.6.
   b) a nucleic acid which is essentially complementary to the nucleic acid of a),
   c) a nucleic acid comprising at least 15 sequential bases of the nucleic acid of a),
   d) a nucleic acid which anneals under stringent hybridisation conditions to the polynucleotide of a), and
   e) a nucleic acid which, but for the degeneracy of the genetic code, would hybridize to the nucleic acid defined in a), b), c) or d).

2. An isolated nucleic acid molecule which is selected from the group comprising
   a) a nucleic acid having at least 70% identity to a nucleic acid sequence set forth in SeqID NO 7, SeqID NO 8, SeqID NO 9 or SeqID NO 10.
   b) a nucleic acid which is complementary to the nucleic acid of a),
   c) a nucleic acid comprising at least 15 sequential bases of the nucleic acid of a),
   d) a nucleic acid which anneals under stringent hybridisation conditions to the nucleic acid of a), and
   e) a nucleic acid which, but for the degeneracy of the genetic code, would hybridize to the nucleic acid defined in a), b), c) or d).

3. The isolated nucleic acid molecule according to claim 1 or 2, whereby the identity is at least 80 %, preferably at least 90 %.

4. The isolated nucleic acid molecule according to claim 1 or 3, whereby the nucleic acid molecule encodes a fibrinogen-binding-protein comprising at least one repeat of an amino acid motive comprising 16 amino acids.

5. The isolated nucleic acid molecule according to claim 4, whereby the encoded fibrinogen-binding protein comprises 19 repeats of the amino acid motive whereby the amino acid motive is the one specified in any of claims 7 and 13.

6. The isolated nucleic acid molecule according to claims 2 or 3, whereby the nucleic acid molecule encodes an adhesion factor which interacts with epithelial cells, preferably human epithelial cells.

7. An isolated nucleic acid molecule encoding for a polypeptide whereby the polypeptide comprises an amino acid motive, whereby the amino acid motive is G-N/S/T-V-L-A/E/M/Q-R-R-X-K/R/W-A/D/E/N/Q-A/F/I/L/V/Y-X-X-K/R-X-X (SEQ ID NO 222).

8. A vector comprising a nucleic acid according to any of claims 1 to 7.

9. A cell comprising the vector according to claim 8.

10. A polypeptide comprising an amino acid sequence, whereby the amino acid sequence is encoded by a nucleic acid molecule according to any one of claims 1 to 6, and fragments of said polypeptide.

11. A polypeptide comprising an amino acid sequence, whereby the amino acid sequence is selected from the group comprising Seq ID NO 11 to 20.

12. A polypeptide comprising an amino acid sequence, whereby the amino acid sequence is selected from the group comprising Seq ID NO 113 to 205.

13. A polypeptide comprising an amino acid motive, whereby the polypeptide comprises an amino acid motive, whereby the amino acid motive is G-N/S/T-V-L-A/E/M/Q-R-R-X-K/R/W-A/D/E/N/Q-A/F/I/L/V/Y-X-X-K/R-X-X (SEQ ID NO 222).

14. A pharmaceutical composition, especially a vaccine, comprising a polypeptide or a fragment thereof, as defined in any one of claims 10 to 13 or a nucleic acid molecule according to any of claims 1 to 7.

15. The pharmaceutical composition according to Claim 14, characterized in that it comprises an immunostimulatory substance, whereby the immunostimulatory substance is preferably selected from the group comprising polycationic polymers, immunostimulatory deoxynucleotides (ODNs), synthetic KLK peptides, neuroactive compounds, alumn, Freund's complete or incomplete adjuvants or combinations thereof.

16. Use of a polypeptide according to any one of the claims 10 to 13 or a fragment thereof for the manufacture of a medicament, especially for the manufacture of a vaccine against bacterial infection.

17. An antibody, or at least an effective part thereof, which binds at least to a selective part of the polypeptide or a fragment thereof according to claims 10 to 13.

18. Use of the antibody according to claim 17 for the preparation of a medicament for treating or preventing bacterial infections, especially *Streptococcus agalactiae* infections.

19. A method for identifying an antagonist capable of reducing or inhibiting the activity of the polypeptide or fragment thereof according to any of the Claim 19 to 24 comprising:

a) contacting an isolated or immobilized polypeptide according to any of the claims 10- 13 or a fragment thereof with a candidate antagonist under

conditions to permit binding of said candidate antagonist to said polypeptide or fragment thereof, in the presence of a component capable of providing a detectable signal in response to the binding of the candidate antagonist to said polypeptide or fragment thereof; and

b) detecting the presence or absence of a signal generated in response to the binding of the antagonist to the polypeptide or fragment thereof, the presence of a signal indicating a compound capable of inhibiting or reducing the activity of the polypeptide or fragment thereof.

20. A method for identifying an antagonist capable of reducing or inhibiting the activity of a polypeptide or a fragment thereof according to any of claims 10 to 13 comprising:

a) providing the polypeptide according to any of the claims 10 to 13 or a fragment thereof,

b) providing an interaction partner of the polypeptide according to any of the claims 10 to 13,

c) providing a candidate antagonist,

d) reacting the polypeptide, the interaction partner of the polypeptide and the candidate antagonist, and

e) determining whether the candidate antagonist inhibits or reduces the activity of the polypeptide.

21. A process for *in vitro* diagnosis of a bacterial infection, preferably *Streptococcus agalactiae* infection, comprising the step of determining the presence of a nucleic acid molecule according to any of the preceding claims, or of a polypeptide according to any of the preceding claims.

22. An affinity device comprising a support material and immobilized to said support material a polypeptide according to any of the preceding claims or a nucleic acid molecule according to any of the preceding claims.

23. Use of a polypeptide according to any of the preceding claims for the isolation and/or purification and/or identification of an interaction partner of said polypeptide.

24. Use of any of the polypeptides according to any of the preceeding claims for the generation of a peptide binding to said polypeptide.

25. The use according to claim 24, whereby the peptide is selected from the group comprising anticalines.

26. Use of a polypeptide according to any of the preceding claims for the manufacture of a functional nucleic acid, whereby the functional nucleic acid is selected from the group comprising aptamers and spiegelmers.

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3
15. Okt. 2002

Abstract

The present invention is related to nucleic acids coding for adhesion factors of group B streptococcus, adhesion factors of group B streptococcus and uses thereof. More particularly, the present invention is related to a polypeptide being such adhesion factors and comprising an amino acid sequence, whereby the amino acid sequence is selected from the group comprising SEQ ID NO 11 to SEQ ID NO 20, and the use of such polypeptide for the manufacture of a vaccine.

Rec'd PCT/PTO 15 APR 2005
10/531659

1
GATCATTAAATAAATCAAGGTTAGTTAGCTTGAAAGATATAAATATATTCCAAAATTCCA
61
AAAAGTAATTGGCATAGTGACAAAAACTATTGCTCCCCTGCTTTAGAAATAATTTATTTT
121
TAATTTAATATTAAAAGTAAACTGAAGAATCTAGTTATATTTAAAAAGTAAAGGTTGCAT
181
TTTAACTAAATTATGTTAAACTACTGTTATGCGATGAGTCGATATGTGGTTTTACCACTA
241
TTGCGCAGGGAGATTATAAACGCAGGAGCGGATCTTGATAAGTTGTGTGAACCTTCTTGT
301
CACACTTGAAAAGGTGCCCTTAGCTTACTACTACTTGTAATTTCTTACAAATTGTGGTAA
361
GTAGCTGAAAAGCAAAAAAGAAAGAACCAGTTTGGTTCTTTCTTTTTTGCATAAATAAGT
421
CACAATTTCCTTCTTAAAATTATGTCTTTACTTAACTTTAATTGAATATGCTACCATCAC
481
ATTCTTTGTAAAATTTTTAAATAATCTAGTTTCTGATGGTTTAGATGAAGTATTAAAAAT
541
ATACTATTACCTCATTGTAAATCTTAATGTTAGTATGACTATCTATCATGCTTTATAATA
601
TTAAAGGAAAATTTAAAAATATCATGTTTTAGATATCAACTATTTAATTTTAAACATACA
661
AATTAATAATAAATTGCAACTAAATAATAAATTATCTTGACATAACTTATAAAATGTTTT
721
AATATATAATCTAAATAAAAGTAATAATAAAATGACTTTTAAAATTTAAAAAAAGTAAGG
RBS
781
AGAAAATTAATTGTTCAATAAAATAGGTTTTAGAACTTGGAAATCAGGAAAGCTTTGGCT
841 *M F N K I G F R T W K S G K L W L*
TTATATGGGAGTGCTAGGATCAACTATTATTTAGGATCAAGTCCTGTATCTGCTATGGA
Y M G V L G S T I I L G S S P V S A M D
901 Repeat 1 (SEQ ID 21)
TAGTGTTGGAAATCAAAGTCAGGGCAATGTTTTAGAGCGTCGTCAACGTGATGCAGAAAA
S V G N Q S Q G N V L E R R Q R D A E N
961 Repeat 2 (SEQ ID 22) Repeat 3 (SEQ ID 23)
CAGAAGCCAAGGCAATGTTCTAGAGCGTCGTCAACGCGATGTTGAGAATAAGAGCCAAGG
R S Q G N V L E R R Q R D V E N K S Q G
1021 Repeat 4 (SEQ ID 24)
CAATGTTTTAGAGCGTCGTCAACGTGATGCGGAAAACAAGAGCCAAGGCAATGTTTTAGA
N V L E R R Q R D A E N K S Q G N V L E
1081 Repeat 5 (SEQ ID 25)
GCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGGCAATGTTCTAGAGCGTCGTCAACG
R R Q R D A E N R S Q G N V L E R R Q R
1141 Repeat 6 (SEQ ID 26)
TGATGCAGAAAACAGAAGCCAAGGCAATGTTCTAGAGCGTCGTCAACGCGATGCAGAAAA

Fig. 1-1

D A E N R S Q G N V L E R R Q R D A E N
1201
Repeat 7 (SEQ ID 27) Repeat 8 (SEQ ID 28)
CAGAAGCCAAGGTAATGTTCTAGAGCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGG
R S Q G N V L E R R Q R D A E N R S Q G
1261
Repeat 9 (SEQ ID 29)
TAATGTTCTAGAGCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGGTAATGTTCTAGA
N V L E R R Q R D A E N R S Q G N V L E
1321
Repeat 10 (SEQ ID 30)
GCGTCGTCAACGCGATGTTGAGAATAAGAGCCAAGGCAATGTTTTAGAGCGTCGTCAACG
R R Q R D V E N K S Q G N V L E R R Q R
1381
Repeat 11 (SEQ ID 31)
TGATGCGGAAAACAAGAGCCAAGGCAATGTTTTAGAGCGTCGTCAACGTGATGCAGAAAA
D A E N K S Q G N V L E R R Q R D A E N
1441
Repeat 12 (SEQ ID 32) Repeat 13 (SEQ ID 33)
CAGAAGCCAAGGCAATGTTTTAGAGCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGG
R S Q G N V L E R R Q R D A E N R S Q G
1501
Repeat 14 (SEQ ID 34)
CAATGTTCTAGAGCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGGCAATGTTCTAGA
N V L E R R Q R D A E N R S Q G N V L E
1561
Repeat 15 (SEQ ID 35)
GCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGGCAATGTTCTAGAGCGTCGTCAACG
R R Q R D A E N R S Q G N V L E R R Q R
1621
Repeat 16 (SEQ ID 36)
CGATGCAGAAAACAGAAGCCAAGGTAATGTTCTAGAGCGTCGTCAACGTGATGCAGAAAA
D A E N R S Q G N V L E R R Q R D A E N
1681
Repeat 17 (SEQ ID 37) Repeat 18 (SEQ ID 38)
CAGAAGCCAAGGCAATGTTTTAGAGCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGG
R S Q G N V L E R R Q R D A E N R S Q G
1741
Repeat 19 (SEQ ID 39)
CAATGTTTTAGAGCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGGCAATGTTTTAGA
N V L E R R Q R D A E N R S Q G N V L E
1801
GCGTCGTCAACGTGATGCGGAAAACAAGAGCCAAGTAGGTCAACTTATAGGGAAAAATCC
R R Q R D A E N K S Q V G Q L I G K N P
1861
ACTTCTTTCAAAGTCAATTATATCTAGAGAAAATAATCACTCGAGTCAAGGTGACTCTAA
L L S K S I I S R E N N H S S Q G D S N
1921
CAAACAGTCATTCTCTAAAAAAGTATCTCAGGTTACTAATGTAGCTAATAGACCGATGTT
K Q S F S K K V S Q V T N V A N R P M L
1981
AACTAATAATTCTAGAACAATTTCAGTGATAAATAAATTACCTAAAACAGGTGATGATCA
T N N S R T I S V I N K L P K T G D D Q
2041

Fig. 1-2

AAATGTCATTTTTAAACTTGTAGGTTTTGGTTTAATTTTGTTAACAAGTCGCTGCGGTTT
N V I F K L V G F G L I L L T S R C G L

2101
GAGACGCAATGAAAATTAAGTATAATCAATCATTTAGTAACTATATATAATGATATATGC
R R N E N *

2161
AATCAATAAAAAGGAATCGGATACGAGATTCCTTTTTATAATTAGGTTGGTTAGGGTGAC

2221
TTTTTTCATTTGGCTATTCTTGAAAGTTTATAAAAATGTAGTTATAATAGTCACATTAAA

2281
ATGTTTTGAAAATATTGATGAACAACATCAACAAATAGAGGTCATTATATGGGATATACC

2341
GTTGCTATCGTAGGTGCTACAGGTGCCGTAGGAACACAAATGATTCGTCAATTAGAACAA

2401
TCGAATTTACCAATAGAACAAGTGAAACTTTTATCATCAAGTCGCTCAGCAGGTAAAATT

2461
TTACATTTTAAAGATGAGGCTATACGTGTTGAAGAGACAACAAAAGAATCATTTTACGAT

2521
GTTGATATTGCCTTGTTTTCAGCTGGTGGATC

Fig. 1-3

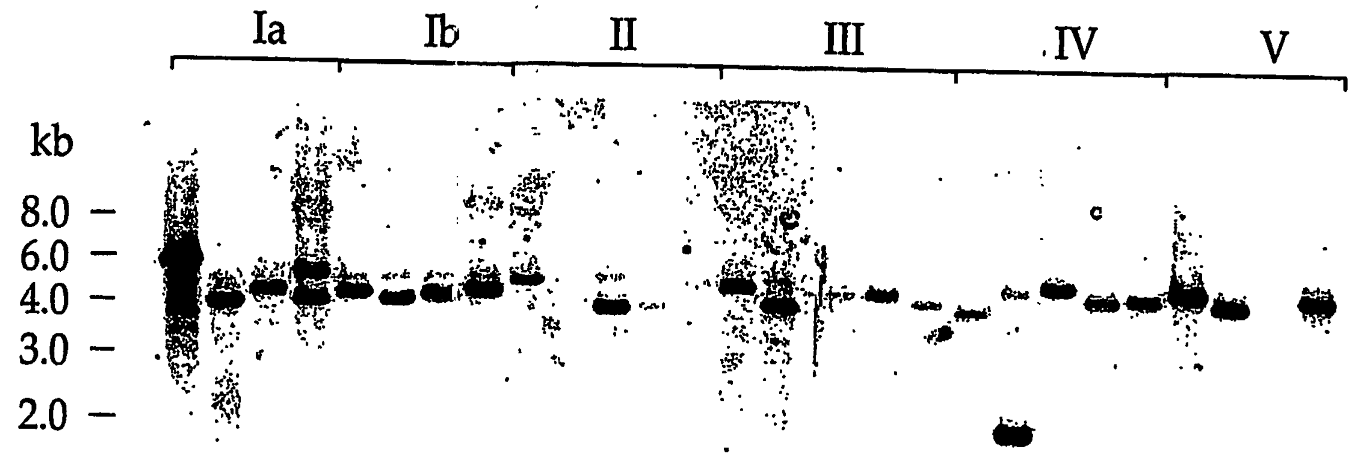

Fig. 2

1
GCATAAATAAGTCACAATTTCCTTCTTAAAATTATGTCTTTACTTAACTTTAATTGAATA
61
TGCTACCATCACATTCTTTGTAAAATTTTTAAATAATCTAGTTTCTGATGGTTTAGATGA
121
AGTATTAAAAATATACTATTACCTCATTGTAAATCTTAATGTTAGTATGACTATCTATCA
181
TGCTTTATAATATTAAAGGAAAATTTAAAAATATCATGTTTTAGATATCAACTATTTAAT
241
TTTAAACATACAAATTAATAATAAATTGCAACTAAATAATAAATTATCTTGACATAACTT
301
ATAAAATGTTTTAATATATAATCTAAATAAAAGTAATAATAAAATGACTTTTAAAATTTA
361
AAAAAAGTAAGGAGAAAATTAATTGTTCAATAAAATAGGTTTTAGAACTTGGAAATCAGG
RBS M F N K I G F R T W K S G
421
AAAGCTTTGGCTTTATATGGGAGTGCTAGGATCAACTATTATTTTAGGATCAAGTTCTGT
K L W L Y M G V L G S T I I L G S S S V
481 Repeat 1 (SEQ ID 40)
ATCTGCTATGGATAGTGTTGGAAATCAAAGTCAGGGCAATGTTTTAGAGCGTCGTCAACG
S A M D S V G N Q S Q G N V L E R R Q R
541 Repeat 2 (SEQ ID 41)
CGATGCAGAAAACAGAAGCCAAGGCAATGTTTTAGAGCGTCGTCAACGTGATGCAGAAAA
D A E N R S Q G N V L E R R Q R D A E N
601 Repeat 3 (SEQ ID 42) Repeat 4 (SEQ ID 43)
CAGAAGCCAAGGCAATGTTTTAGAGCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGG
R S Q G N V L E R R Q R D A E N R S Q G
661 Repeat 5 (SEQ ID 44)
TAATGTTCTAGAGCGTCGTCAACGCGATGTTGAAAATAAAAGCCAAGGCAATGTTTTAGA
N V L E R R Q R D V E N K S Q G N V L E
721 Repeat 6 (SEQ ID 45)
GCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGGTAATGTTCTAGAGCGTCGTCAACG
R R Q R D A E N R S Q G N V L E R R Q R
781 Repeat 7 (SEQ ID 46)
CGATGTTGAAAATAAAAGCCAAGGCAATGTTTTAGAGCGTCGTCAACGTGATGCAGAAAA
D V E N K S Q G N V L E R R Q R D A E N
841 Repeat 8 (SEQ ID 47) Repeat 9 (SEQ ID 48)
CAGAAGCCAAGGTAATGTTCTAGAGCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGG
R S Q G N V L E R R Q R D A E N R S Q G
901 Repeat 10 (SEQ ID 49)
CAATGTTTTAGAGCGTCGTCAACGCGATGCAGAAAACAGAAGCCAAGGCAATGTTCTAGA
N V L E R R Q R D A E N R S Q G N V L E

Fig. 3-1

961

Repeat 11 (SEQ ID 50)

GCGTCGTCAACGTGATGCTGAAAACAAAAGCCAAGGCAATGTTTTAGAGCGTCGTCAACG

R R Q R D A E N K S Q G N V L E R R Q R

1021 Repeat 12 (SEQ ID 51)

TGATGCAGAAAACAGAAGCCAAGGCAATGTTTTAGAGCGTCGTCAACGTGATGCTGAAAA

D A E N R S Q G N V L E R R Q R D A E N

1081 Repeat 13 (SEQ ID 52) Repeat 14 (SEQ ID 53)

CAGAAGCCAAGGCAATGTTTTAGAGCGTCGTCAACGCGATGCAGAAAACAGAAGCCAAGG

R S Q G N V L E R R Q R D A E N R S Q G

1141 Repeat 15 (SEQ ID 54)

TAATGTTCTAGAGCGTCGTCAACGTGATGCGGAAAACAAGAGCCAAGGCAATGTTTTAGA

N V L E R R Q R D A E N K S Q G N V L E

1201 Repeat 16 (SEQ ID 55)

GCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGGCAATGTTTTAGAGCGTCGTCAACG

R R Q R D A E N R S Q G N V L E R R Q R

1261 Repeat 17 (SEQ ID 56)

CGATGTTGAGAATAAGAGCCAAGGCAATGTTTTAGAGCGTCGTCAACGTGATGCGGAAAA

D V E N K S Q G N V L E R R Q R D A E N

1321

CAAGAGCCAAGTAGGTCAACTTATAGGGAAAAATCCACTTCTTTCAAAGTCAATTATATC

K S Q V G Q L I G K N P L L S K S I I S

1381

TAGAGAAAATAATCACTCTAGTCAAGGTGACTCTAACAAACAGTCATTCTCTAAAAAAGT

R E N N H S S Q G D S N K Q S F S K K V

1441

ATCTCAGGTTACTAATGTAGCTAATAGACCGATGTTAACTAATAATTCTAGAACAATTTC

S Q V T N V A N R P M L T N N S R T I S

1501

AGTGATAAATAAATTACCTAAAACAGGTGATGATCAAAATGTCATTTTTAAACTTGTAGG

V I N K L P K T G D D Q N V I F K L V G

1561

TTTTGGTTTAATTTTGTTAACAAGTCGCTGCGGTTTGAGACGCAATGAAAATTAAGTATA

F G L I L L T S R C G L R R N E N *

1621

ATCAATCATTTAGTAACTATATATAATGATATATGCAATCAATAAAAAGGAATCGGATAC

GAGATTCCTTTTTATAATTAGGTTGGTTAGGGTGACTTTTTCATTTGGCTATTCTTGAA

1741 1761 1781

AGTTTATAAAAATGTAGTATAATAGTCACATTAAAATGTTTTGAAAATATTGATGAACAA

1801

CATCAACAAATAGAGGTCAT

Fig. 3-2

1
GCATAAATAAGTCACAATTTCCTTCTAAAAATTATGTCTTTACTTAACTTTAATTGAATA
61
TGCTACCATCACATTCTTTGTAAAATTTTTAAATAATCTAGTTTCTGATGGTTTAGATGA
121
AGTATTAAAAATATACTATTATCTCATTGTAAATCCTAATGTTAGTATGACTATCTATCA
181
TGTTTTATAATATTGAAGGAAAATTTAAAAATATCATGTTTTAGATATCAACTATTTAAT
241
TTTAAACATACAAATTAATAATAAATTGCAATTAAATAACAAATTACCTTGACATAAATT
301
ATAAAATGTTTTAATATATATAATCTAAATAAAAATAATAATAAAATGACTTTTAAAATT
361
TAAAAAAAGTAAGGAGAAAATTAATTGTTCAATAAAATAGGTTTTAGAACTTGGAAATCA
421 RBS *M F N K I G F R T W K S*
GGAAAGCTTTGGCTTTATATGGGAGTGCTAGGATCAACTATTATTTTAGGATCAAGTCCT
G K L W L Y M G V L G S T I I L G S S P
481 Repeat 1 (SEQ ID 57)
GTATCTGCTATGGATAGTGTTGGAAATCAAAGTCAAGGTAATGTTCTAGAGCGTCGTCAA
V S A M D S V G N Q S Q G N V L E R R Q
541 Repeat 2 (SEQ ID 58)
CGTGATGCGGATAACAAGAGCCAAGGCAATGTTCTAGAACGTCGTCAACGCGATGTAGAA
R D A D N K S Q G N V L E R R Q R D V E
601 Repeat 3 (SEQ ID 59)
AACAGAAGCCAAGGCAATGTTCTAGAGCGTCGTCAACGCGATGCGGATAACAAGAGCCAA
N R S Q G N V L E R R Q R D A D N K S Q
Repeat 4 (SEQ ID 60) Repeat 5 (SEQ ID 61)
GGCAATGTTTTAGAGCGCCGCCAACGCGATGCAGAAAACAAAAGTCAGGGCAATGTTCTA
G N V L E R R Q R D A E N K S Q G N V L
721 Repeat 6 (SEQ ID 62)
GAACGTCGTCAACGTGATGTTGAGAATAAGAGCCAAGGCAATGTTCTAGAGCGTCGCCAA
E R R Q R D V E N K S Q G N V L E R R Q
781 Repeat 7 (SEQ ID 63)
CGTGATGCAGAAAACAAAAGTCAGGGTAATGTTCTAGAGCGTCGTCAACGCGATGCAGAT
R D A E N K S Q G N V L E R R Q R D A D
841 Repeat 8 (SEQ ID 64)
AACAAGAGCCAAGGTAATGTTCTAGAACGTCGTCAACGCGATGTGGAAAACAAAAGTCAG
N K S Q G N V L E R R Q R D V E N K S Q
Repeat 9 (SEQ ID 65) Repeat 10 (SEQ ID 66)
GGCAATGTTCTAGAACGTCGTCAACGTGATGTTGAGAATAAGAGCCAAGGCAATGTTCTA
G N V L E R R Q R D V E N K S Q G N V L
961 Repeat 11 (SEQ ID 67)
GAGCGTCGCCAACGTGATGCAGAAAACAAAAGTCAGGGTAATGTTCTAGAGCGTCGTCAA
E R R Q R D A E N K S Q G N V L E R R Q

Fig. 4-1

1021 Repeat 12 (SEQ ID 68)
CGCGATGCAGATAACAAGAGCCAAGGTAATGTTCTAGAACGTCGTCAACGCGATGTGGAA
R D A D N K S Q G N V L E R R Q R D V E
1081 Repeat 13 (SEQ ID 69)
AACAAAAGTCAGGGCAATGTTCTAGAGCGTCGCCAACGTGATGTTGAGAACAAGAGCCAA
N K S Q G N V L E R R Q R D V E N K S Q
1141
GTAGGTCAACTTATAGGGAAAAATCCACTTCTTTCAAAGTCAACTATATCTAGAGAAAAT
V G Q L I G K N P L L S K S T I S R E N
1201
AATCACTCTAGTCAAGGTGACTCTAACAAACAGTCATTCTCTAAAAAAGTATCTCAGGTT
N H S S Q G D S N K Q S F S K K V S Q V
1261
ACTAATGTAGCTAATAGACCAATGTTAACTAATAATTCTAGAACAATTTCAGTGATAAAT
T N V A N R P M L T N N S R T I S V I N
1321
AAATTACCTAAAACAGGTGATGATCAAAATGTCATTTTTAAACTTGTAGGTTTTGGTTTA
K L P K T G D D Q N V I F K L V G F G L
1381
ATTTTGTTAACAAGTCGCTGCGGTTTGAGACGCAATGAAAATTAAGTATAATCAATCATT
I L L T S R C G L R R N E N *
1441
TAGTAACTATTATAATGATATATGCAATCAATAAAAAGGAATCGGATACAAGATTCCTTT
TTATAATTAGGTTGGTTAGGGTGACTTTTTCATTTGGCTATTCTTGAAAGTTTATAAAAA
1561
TGTAGTATAATAGTCACATTAAAATGTTTTGAAAATATTGATGAACAACATCAACAAATA
1621
GAGGTCAT

Fig. 4-2

1
GCATAAATAAGTCACCAATTTCCCTTCTTAAAATTATGTCTTTACTTAACTTTAATTGAA
61
TATGCTACCATCACATTCTTTGTAAAATTTTTAAATAATCTAGTTTCTGATGGTTTAGAT
121
GAAGTATTAAAAATATACTATTACCTCATTGTAAATCTTAATGTTAGTATGACTATCTAT
181
CATGCTTTATAATATTAAAGGAAAATTTAAAAATATCATGTTTTAGATATCAACTATTTA
241
ATTTTAAACATACAAATTAATAATAAATTGCAACTAAATAATAAATTATCTTGACATAAC
301
TTATAAAATGTTTTAATATATAATCTAAATAAAAGTAATAATAAAATGACTTTTAAAATT
361
TAAAAAAAGTAAGGAGAAAATTAATTGTTCAATAAAATAGGTTTTAGAACTTGGAAATCA
421 RBS M F N K I G F R T W K S
GGAAAGCTTTGGCTTTATATGGGAGTGCTAGGATCAACTATTATTTTAGGATCAAGTCCT
G K L W L Y M G V L G S T I I L G S S P
481 Repeat 1 (SEQ ID 70)
GTATCTGCTATGGATAGTGTTGGAAATCAAAGTCAGGGCAATGTTTTAGAGCGTCGTCAA
V S A M D S V G N Q S Q G N V L E R R Q
541 Repeat 2 (SEQ ID 71)
CGCGATGCAGAAAACAGAAGCCAAGGTAATGTTCTAGAGCGTCGTCAACGCGATGCAGAA
R D A E N R S Q G N V L E R R Q R D A E
601 Repeat 3 (SEQ ID 72)
AACAGAAGCCAAGGTAATGTTCTAGAGCGTCGTCAACGTGATGCGGAAAACAAGAGCCAA
N R S Q G N V L E R R Q R D A E N K S Q
661
GTAGGTCAACTTATAGGGAAAAATCCACTTCTTTCAAAGTCAATTATATCTAGAGAAAAT
V G Q L I G K N P L L S K S I I S R E N
721
AATCACTCTAGTCAAGGTGACTCTAACAAACAGTCATTCTCTAAAAAAGTATCTCAGGTT
N H S S Q G D S N K Q S F S K K V S Q V
781
ACTAATGTAGCTAATAGACCGATGTTAACTAATAATTCTAGAACAATTTCAGTGATAAAT
T N V A N R P M L T N N S R T I S V I N
841
AAATTACCTAAAACAGGTGATGATCAAAATGTCATTTTTAAACTTGTAGGTTTTGGTTTA
K L P K T G D D Q N V I F K L V G F G L
901
ATTTTGTTAACAAGTCGCTGCGGTTTGAGACGCAATGAAAATTAAGTATAATCAATCATT
I L L T S R C G L R R N E N *
961
TAGTAACTATATATAATGATATATGCAATCAATAAAAAGGAATCGGATACGAGATTCCTT

TTTATAATTAGGTTGGTTAGGGTGACTTTTTTCATTTGGCTATTCTTGAAAGTTTATAAA
1081
AATGTAGTATAATAGTCACATTAAAATGTTTTGAAAATATTGATGAACAACATCAACAAA
1141
TAGAGGTCAT

Fig. 5

1
GCATAAATAAGTCACAATTTCCTTCTTAAAATTATGTCTTTACTTAACTTTAATTGAATA
61
TGCTACCATCACATTCTTTGTAAAATTTTTAAATAATCTAGTTTCTGATGGTTTAGATGA
121
AGTATTAAAAATATACTATTACCTCATTGTAAATCTTAATGTTAGTATGACTATCTATCA
181
TGCTTTATAATATTAAAGGAAAATTTAAAAATATCATGTTTTAGATATCAACTATTTAAT
241
TTTAAACATACAAATTAATAATAAATTGCAACTAAATAATAAATTATCTTGACATAACTT
301
ATAAAATGTTTTAATATATAATCTAAATAAAAGTAATAATAAAATGACTTTTAAAATTTA
361
AAAAAAGTAAGGAGAAAATTAATTGTTCAATAAAATAGGTTTTAGAACTTGGAAATCAGG
421 RBS *M F N K I G F R T W K S G*
AAAGCTTTGGCTTTATATGGGAGTGCTAGGATCAACTATTATTTTAGGATCAAGTCCTGT
K L W L Y M G V L G S T I I L G S S P V
481 Repeat 1 (SEQ ID 73)
ATCTGCTATGGATAGTGTTGGAAATCAAAGCCAAGGCAATGTTCTAGAGCGTCGTCAACG
S A M D S V G N Q S Q G N V L E R R Q R
541 Repeat 2 (SEQ ID 74)
CGATGCAGAAAACAGAAGCCAAGGTAATGTTTTAGAACGTCGTCAACGCGATGTTGAGAA
D A E N R S Q G N V L E R R Q R D V E N
601 Repeat 3 (SEQ ID 75) Repeat 4 (SEQ ID 76)
CAAGAGCCAAGGTAATGTTTTAGAGCGTCGCCAACGTGATGCGGAAAACAAAAGTCAGGG
K S Q G N V L E R R Q R D A E N K S Q G
661 Repeat 5 (SEQ ID 77)
CAATGTTTTAGAGCGTCGTCAACGTGATGCAGAAAACAGAAGCCAAGGTAATGTTCTAGA
N V L E R R Q R D A E N R S Q G N V L E
721 Repeat 6 (SEQ ID 78)
GCGTCGTCAACGCGATGTTGAGAATAAGAGCCAAGGCAATGTTCTAGAGCGTCGTCAACG
R R Q R D V E N K S Q G N V L E R R Q R
781 Repeat 7 (SEQ ID 79)
CGATGTTGAGAATAAGAGCCAAGGTAATGTTCTAGAGCGTCGTCAACGCGATGTTGAGAA
D V E N K S Q G N V L E R R Q R D V E N
841 Repeat 8 (SEQ ID 80) Repeat 9 (SEQ ID 81)
TAAGAGCCAAGGTAATGTTCTAGAGCGTCGTCAACGTGATGCGGAAAACAAGAGCCAAGG
K S Q G N V L E R R Q R D A E N K S Q G
901 Repeat 10 (SEQ ID 82)
CAATGTTCTAGAGCGTCGTCAACGCGATGCAGAAAACAGAAGCCAAGGTAATGTTTTAGA
N V L E R R Q R D A E N R S Q G N V L E
961
GCGTCGCCAACATGATGTTGAGAATAAGAGTCAAGTAGGTCAACTTATAGGGAAAAATCC
R R Q H D V E N K S Q V G Q L I G K N P
1021
ACTTTTTTCAAAGTCAACTGTATCTAGAGAAAATAATCACTCTAGTCAAGGTGACTCTAA
L F S K S T V S R E N N H S S Q G D S N
1081

Fig. 6-1

CAAACAGTCATTCTCTAAAAAAGTATCTCAGGTTACTAATGTAGCTAATAGACCGATGTT
K Q S F S K K V S Q V T N V A N R P M L

1141
AACTAATAATTCTAGAACAATTTCAGTGATAAATAAATTACCTAAAACAGGTGATGATCA
T N N S R T I S V I N K L P K T G D D Q

1201
AAATGTCATTTTTAAACTTGTAGGTTTTGGTTTAATTTTATTAACAAGTCTCTGCGGTTT
N V I F K L V G F G L I L L T S L C G L

1261
GAGACGCAATGAAAATTAAGTATAATCAACCATTTAGTAACTATTATAATGATATATGCA
R R N E N *

1321
ATCAATAAAAAAGGAATCGAATACGAGATTCCTTTTTATAATTAGGTTGGTTAGGGTGAC

1381
TTTTTTCATTTGGCTATTCTTGAAAGTTTATAAAAATGTAGTATAATAGTCACATTAAAA

1441
TGTTTTGAAAATATTGATGAACAACATCATCAAATAGAGGTCAT

Fig. 6-2

1

GCATAAATAAGTCACAATTTCCTTCTAAAAATTATGTCTTTACTTAACTTTAATTGAATA

61

TGCTACCATCACATTCTTTGTAAAATTTTTAAATAACCTAGTTTCTGATGGTTTAGATGA

121

AGTATTAAAAATATACTATTATCTCATTGTAAATCCTAATGTTAGTATGACTATCTATCA

181

TGTTTTATAATATTGAAGGAAAATTTAAAAATATCATGTTTTAGATATCAACTATTTAAT

241

TTTAAACATACAAATTAATAATAAATTGCAATTAAATAACAAATTACCTTGACATAAATT

301

ATAAAATGATTTAATATATATAATCTAAATAAAAATAATAATAAAATGACTTTTAAAATT

361

TAAAAAAAGTAAGGAGAAAATTAATTGTTCAATAAAATAGGTTTTAGAACTTGGAAATCA

421 RBS *M F N K I G F R T W K S*

GGAAAGCTTTGGCTTTATATGGGAGTGCTAGGATCAACTATTATTTTAGGATCAAGTCCT

G K L W L Y M G V L G S T I I L G S S P

481

Repeat 1 (SEQ ID 83)

GTATCTGCTATGGATAGTGTTGGAAATCAAAGTCAAGGTAATGTTCTAGAGCGTCGCCAA

V S A M D S V G N Q S Q G N V L E R R Q

541

Repeat 2 (SEQ ID 84)

CGTGATGCGGATAACAAGAGCCAAGGTAATGTTTTAGAGCGTCGCCAACGTGATGCAGAT

R D A D N K S Q G N V L E R R Q R D A D

601

Repeat 3 (SEQ ID 85)

AACAAAAGTCAGGGCAATGTTCTAGAACGTCGCCAACGTGATGTTGATAACAAGAGCCAA

N K S Q G N V L E R R Q R D V D N K S Q

Repeat 4 (SEQ ID 86) Repeat 5 (SEQ ID 87)

GGTAACGTTCTAGAGCGTCGCCAACGCGATGCTGATAACAAGAGCCAAGGTAATGTTTTA

G N V L E R R Q R D A D N K S Q G N V L

721

Repeat 6 (SEQ ID 88)

GAGCGCCGCCAACGCGATGCAGATAACAAAAGTCAAGGTAATGTTCTAGAGCGTCGCCAA

E R R Q R D A D N K S Q G N V L E R R Q

781

Repeat 7 (SEQ ID 89)

CGCGATGTTGATAACAAGAGCCAGGGTAATGTTTTAGAGCGTCGCCAACGCGATGCAGAT

R D V D N K S Q G N V L E R R Q R D A D

841

Repeat 8 (SEQ ID 90)

AACAAAAGTCAGGGTAATGTTTTAGAGCGTCGCCAACGCGATGTTGATAACAAAAGCCAA

N K S Q G N V L E R R Q R D V D N K S Q

Repeat 9 (SEQ ID 91) Repeat 10 (SEQ ID 92)

GGTAATGTTTTAGAGCGTCGCCAACGTGATGCTGATAACAAAAGTCAGGGCAATGTTCTA

G N V L E R R Q R D A D N K S Q G N V L

961

Repeat 11 (SEQ ID 93)

GAGCGTCGCCAACGTGATGCGGATAACAAAAGCCAAGGTAATGTTCTAGAGCGTCGCCAA

E R R Q R D A D N K S Q G N V L E R R Q

1021

Repeat 12 (SEQ ID 94)

CGCGATGCGGATAACAAAAGTCAGGGCAATGTTTTAGAGCGTCGCCAACGTGATGCTGAT

R D A D N K S Q G N V L E R R Q R D A D

Fig. 7-1

1081 Repeat 13 (SEQ ID 95)

AACAAAAGTCAAGGTAATGTTCTAGAGCGTCGCCAACGCGATGCAGATAACAAAAGCCAA
N K S Q G N V L E R R Q R D A D N K S Q

Repeat 14 (SEQ ID 96) Repeat 15 (SEQ ID 97)

GGTAATGTTCTAGAGCGTCGCCAACGCGATGCTGATAACAAAAGTCAAGGTAATGTTCTA
G N V L E R R Q R D A D N K S Q G N V L

1201 Repeat 16 (SEQ ID 98)

GAGCGTCGCCAACGTGATGCTGATAACAAGAGCCAAGGCAATGTTCTTGAGCGTCGTCAA
E R R Q R D A D N K S Q G N V L E R R Q

1261 Repeat 17 (SEQ ID 99)

CGCGATGTCGATAACAAAAGTCAGGGTAATGTTTTAGAGCGTCGCCAACGTGATGCGGAT
R D V D N K S Q G N V L E R R Q R D A D

1321 Repeat 18 (SEQ ID 100)

AACAAGAGTCAAGGTAATGTTTTAGAGCGTCGCCAACGCGATGCGGATAACAAGAGCCAA
N K S Q G N V L E R R Q R D A D N K S Q

Repeat 19 (SEQ ID 101) Repeat 20 (SEQ ID 102)

GGTAATGTTTTAGAGCGTCGCCAACGCGATGCGGATAACAAGAGTCAAGGTAATGTTTTA
G N V L E R R Q R D A D N K S Q G N V L

1441 Repeat 21 (SEQ ID 103)

GAGCGTCGCCAACGCGATGCGGATAACAAGAGCCAAGGTAATGTTTTAGAGCGTCGCCAA
E R R Q R D A D N K S Q G N V L E R R Q

1501 Repeat 22 (SEQ ID 104)

CGCGATGCAGATAACAAAAGTCAAGGTAATGTTTTAGAGCGTCGCCAACGCGATGCTGAT
R D A D N K S Q G N V L E R R Q R D A D

1561 Repeat 23 (SEQ ID 105)

AACAAGAGCCAAGGTAATGTTTTAGAGCGTCGTCAACGTGATGCAGATAACAAAAGTCAG
N K S Q G N V L E R R Q R D A D N K S Q

Repeat 24 (SEQ ID 106) Repeat 25 (SEQ ID 107)

GGCAATGTTTTAGAGCGTCGTCAACGTGATGCGGATAACAAGAGCCAAGGTAATGTTTTA
G N V L E R R Q R D A D N K S Q G N V L

1681 Repeat 26 (SEQ ID 108)

GAGCGTCGCCAACGTGATGCGGATAACAAGAGCCAGGGCAATGTTCTAGAACGTCGTCAA
E R R Q R D A D N K S Q G N V L E R R Q

1741 Repeat 27 (SEQ ID 109)

CGTGATGCGGATAACAAGAGCCAAGGTAACGTTTTAGAGCGTCGCCAACGTGATGCGGAT
R D A D N K S Q G N V L E R R Q R D A D

1801 Repeat 28 (SEQ ID 110)

AACAAGAGCCAGGGCAATGTTTTAGAGCGCCGCCAACGCGATGCAGATAACAAAAGTCAA
N K S Q G N V L E R R Q R D A D N K S Q

Repeat 29 (SEQ ID 111) Repeat 30 (SEQ ID 112)

GGTAATGTTCTAGAGCGTCGCCAACGCGATGCAGATAACAAGAGCCAGGGTAATGTTCTA
G N V L E R R Q R D A D N K S Q G N V L

1921

GAGCGTCGCCAACGCGATGCGGAAAACAAAAGTCAAGTAGGTCAACTTATAGGGAAAAAT
E R R Q R D A E N K S Q V G Q L I G K N

1981

Fig. 7-2

```
CCACTTTTTTCAAAGTCAACTGTATCTAGAGAAAATAATCACTCTAGTCAAGGTGACTCT
 P  L  F  S  K  S  T  V  S  R  E  N  N  H  S  S  Q  G  D  S
2041
AACAAACAGTCATTCTCTAAAAAAATATCTCAGGTTACTAATGTAGCTAATGGACCGATG
 N  K  Q  S  F  S  K  K  I  S  Q  V  T  N  V  A  N  G  P  M
2101
TTAACTAATAATTCTAGAACAATTTCAGTGATAAATAAATTACCTAAAACAGGTGATGAT
 L  T  N  N  S  R  T  I  S  V  I  N  K  L  P  K  T  G  D  D
2161
CAAAATGTCATTTTTAAACTTGTAGGTTTTGGTTTAATTTTGTTAACAAGTCTCTGCGGT
 Q  N  V  I  F  K  L  V  G  F  G  L  I  L  L  T  S  L  C  G
2221
TTGAGACGCAATGAAAATTAAGTATAATCAACCATTTAGTAACTATTATAATGATATATG
 L  R  R  N  E  N  *
2281
CAATCAATAAAAAAGGAATCGAATACGAGATTCCTTTTTATAATTAGGTTGGTTAGGGTG
2341                2361                2381
ACTTTTTTCATTTGGCTATTCTTGAAAGTTTATAAAAATGTAGTATAATAGTCACATTAA
2401                2421                2441
AATGTTTTGAAAATATTGATGAACAACATCATCAAATAGAGGTCAT
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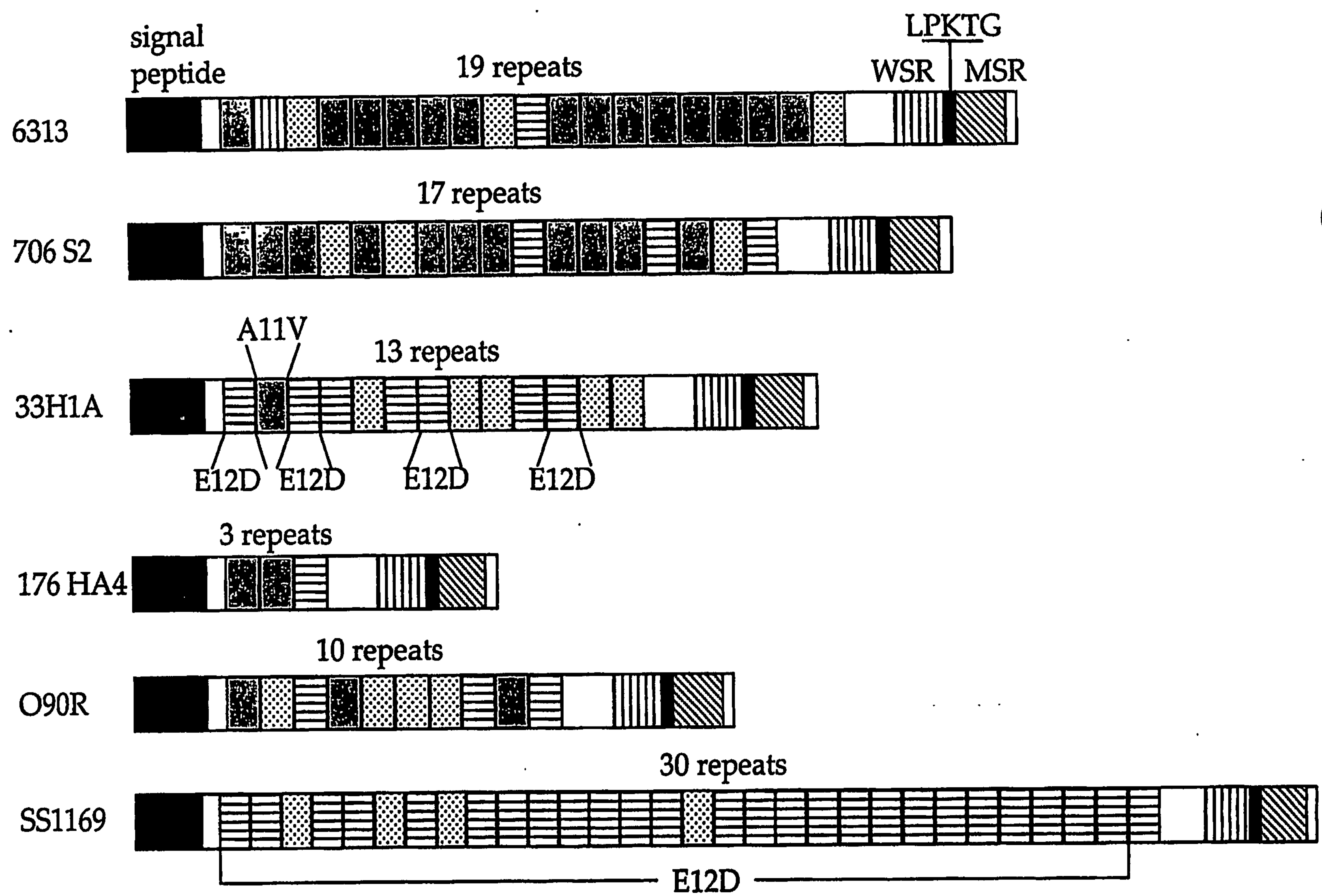
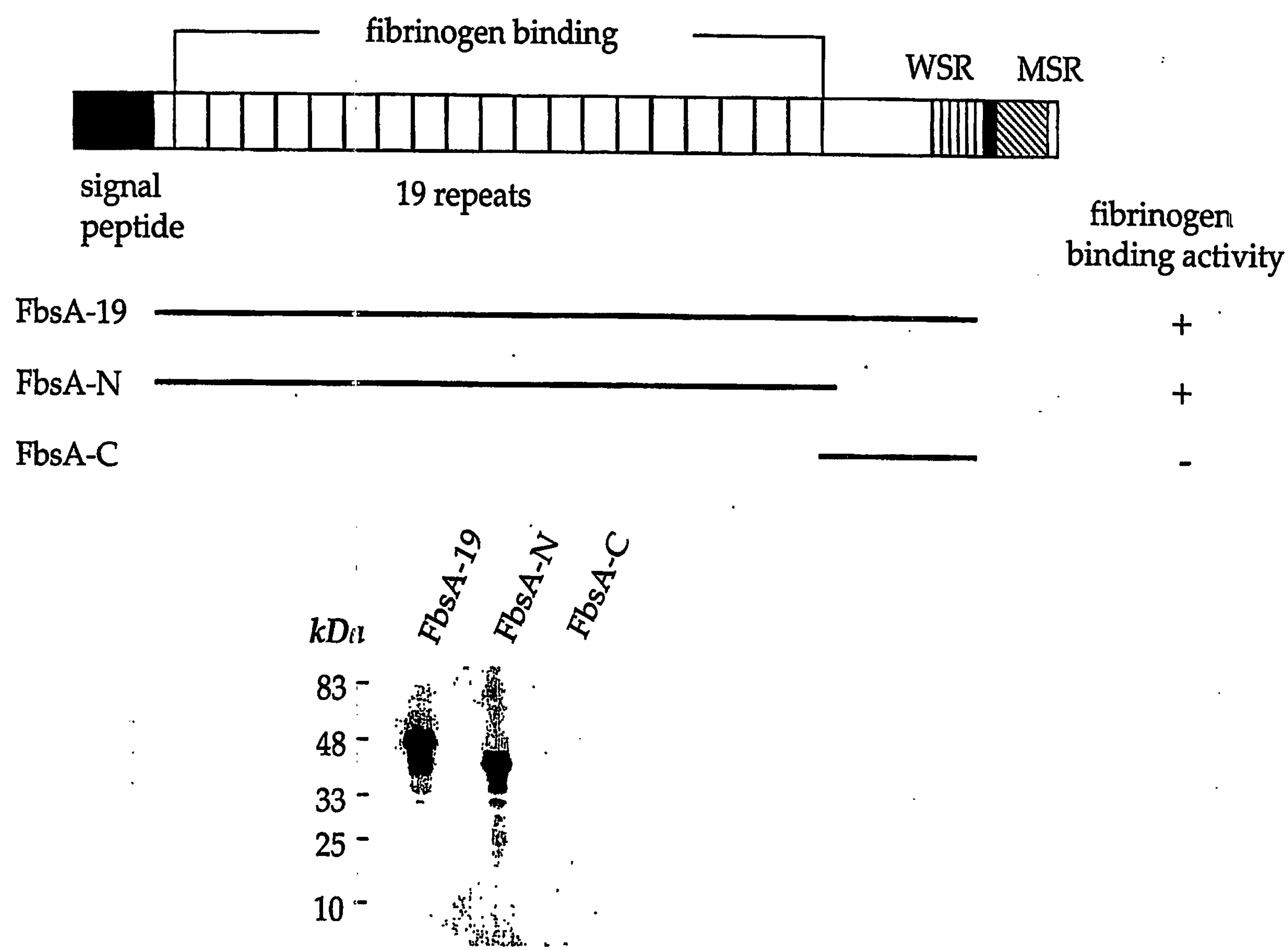
Fig. 7-3



signal
peptide
19 repeats
LPKTG
WSR
MSR
6313
17 repeats
706 S2
A11V
13 repeats
33H1A
E12D
E12D
E12D
E12D
3 repeats
176 HA4
10 repeats
O90R
30 repeats
SS1169
E12D

Fig. 8



fibrinogen binding
WSR
MSR
signal peptide
19 repeats
fibrinogen binding activity
FbsA-19
+
FbsA-N
+
FbsA-C
-
kDa
83
48
33
25
10
FbsA-19
FbsA-N
FbsA-C

Fig. 9

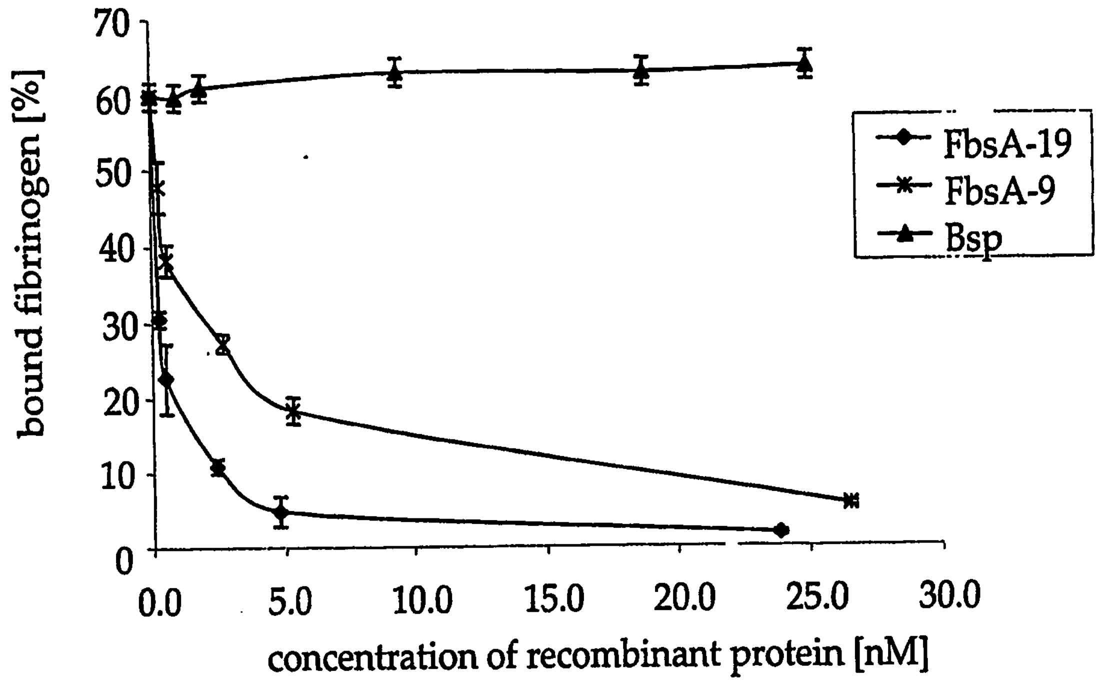
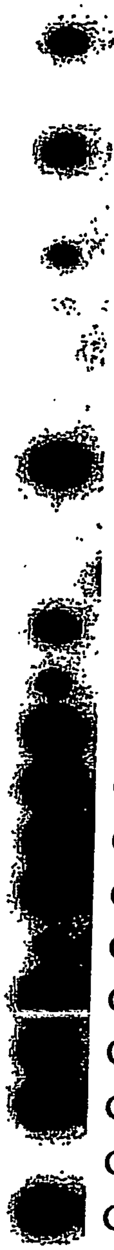

70
60
50
40
30
20
10
0
bound fibrinogen [%]
0.0
5.0
10.0
15.0
20.0
25.0
30.0
concentration of recombinant protein [nM]
FbsA-19
FbsA-9
Bsp

Fig. 10



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GNALERRQRDAENRSQ (SeqID 208)
GNVAERRQRDAENRSQ (SeqID 209)
GNVLARRQRDAENRSQ (SeqID 210)
GNVLEARQRDAENRSQ (SeqID 211)
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GNVLERRQADAENRSQ (SeqID 214)
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GNVLERRQRDAANRSQ (SeqID 217)
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GNVLERRQRDAENASQ (SeqID 219)
GNVLERRQRDAENRAQ (SeqID 220)
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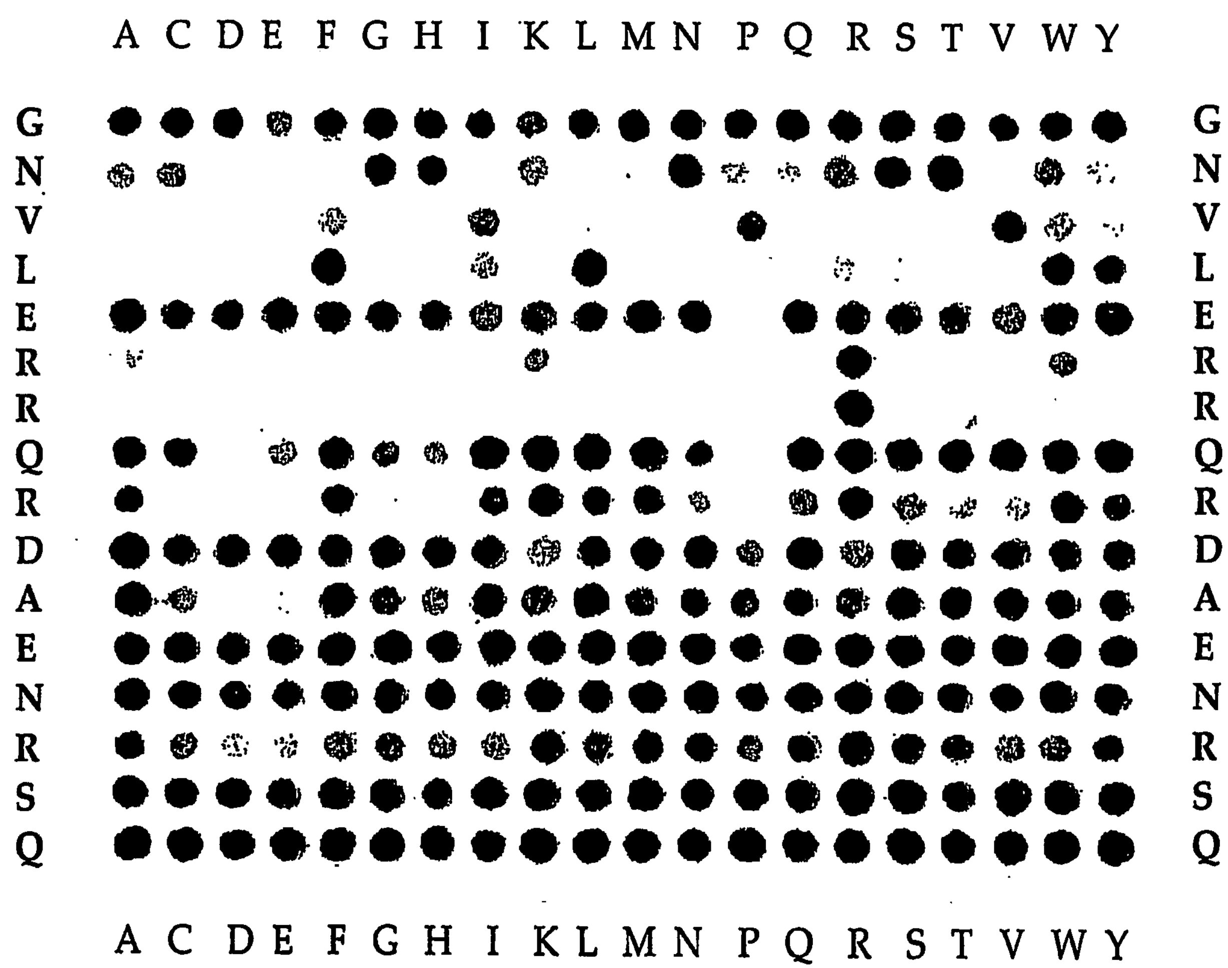
Fig. 11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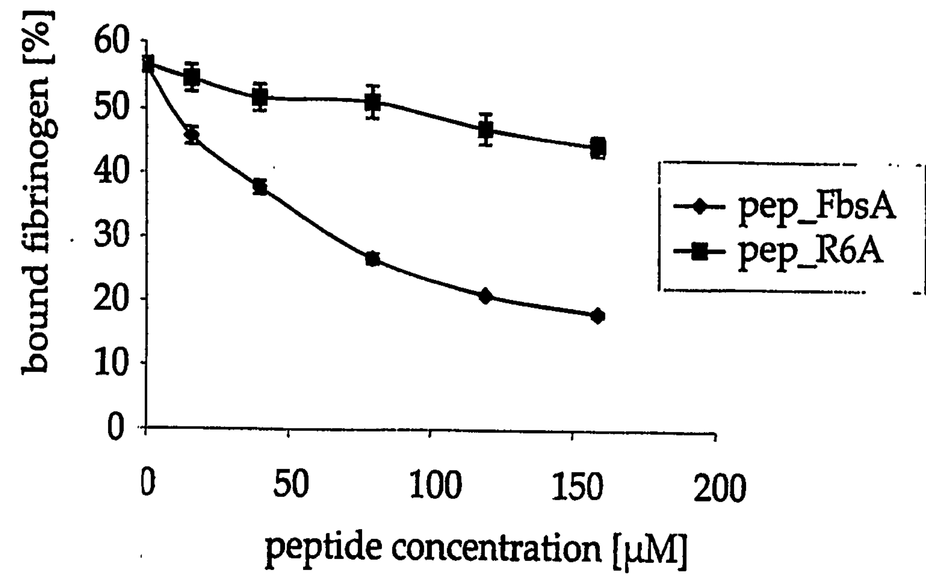
G N V L E R R Q R D A E N R S Q
A C D E F G H I K L M N P Q R S T V W Y

Fig. 12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60
50
40
30
20
10
0
0
50
100
150
200
peptide concentration [μM]
pep_FbsA
pep_R6A

Fig. 13

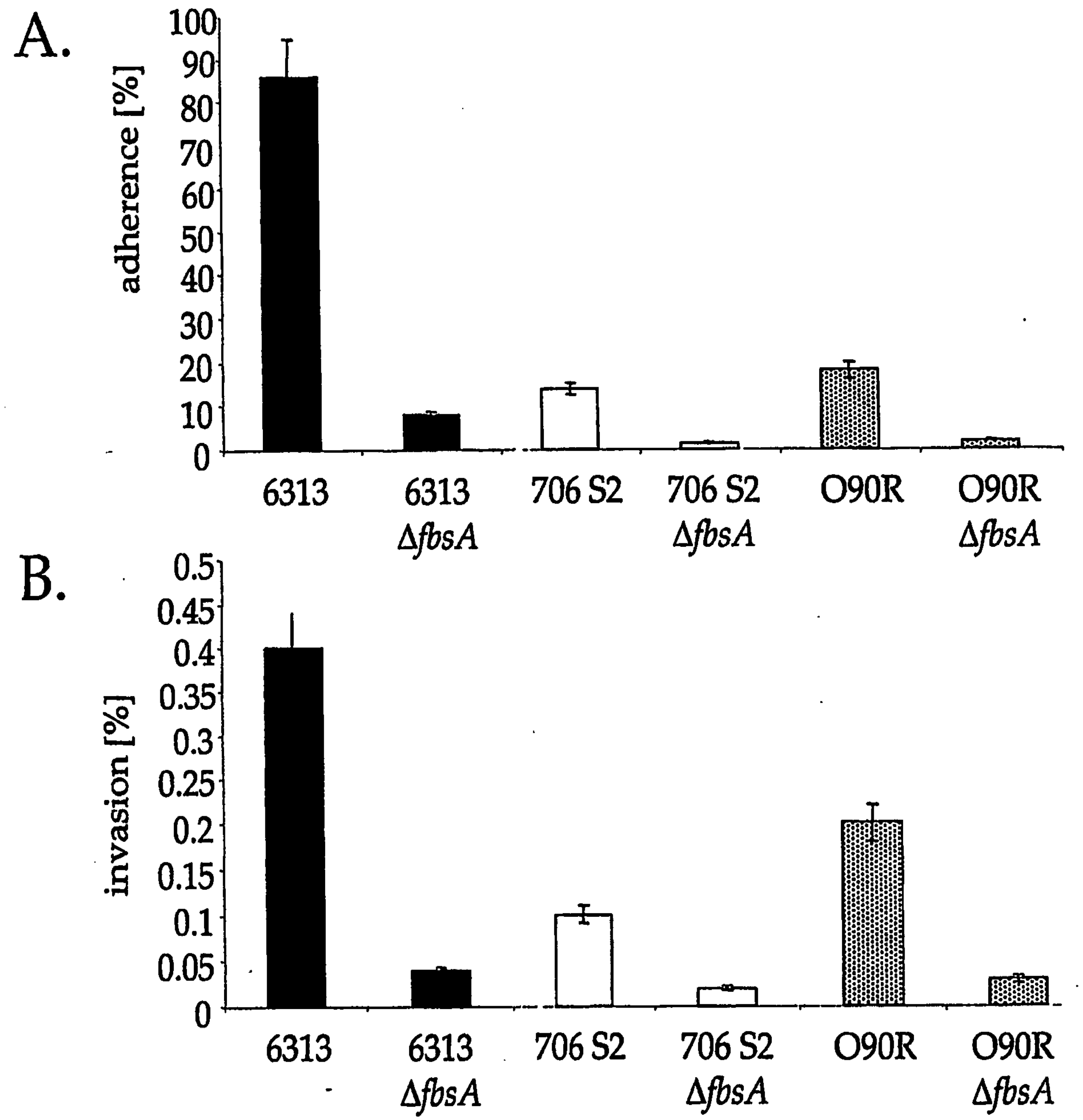
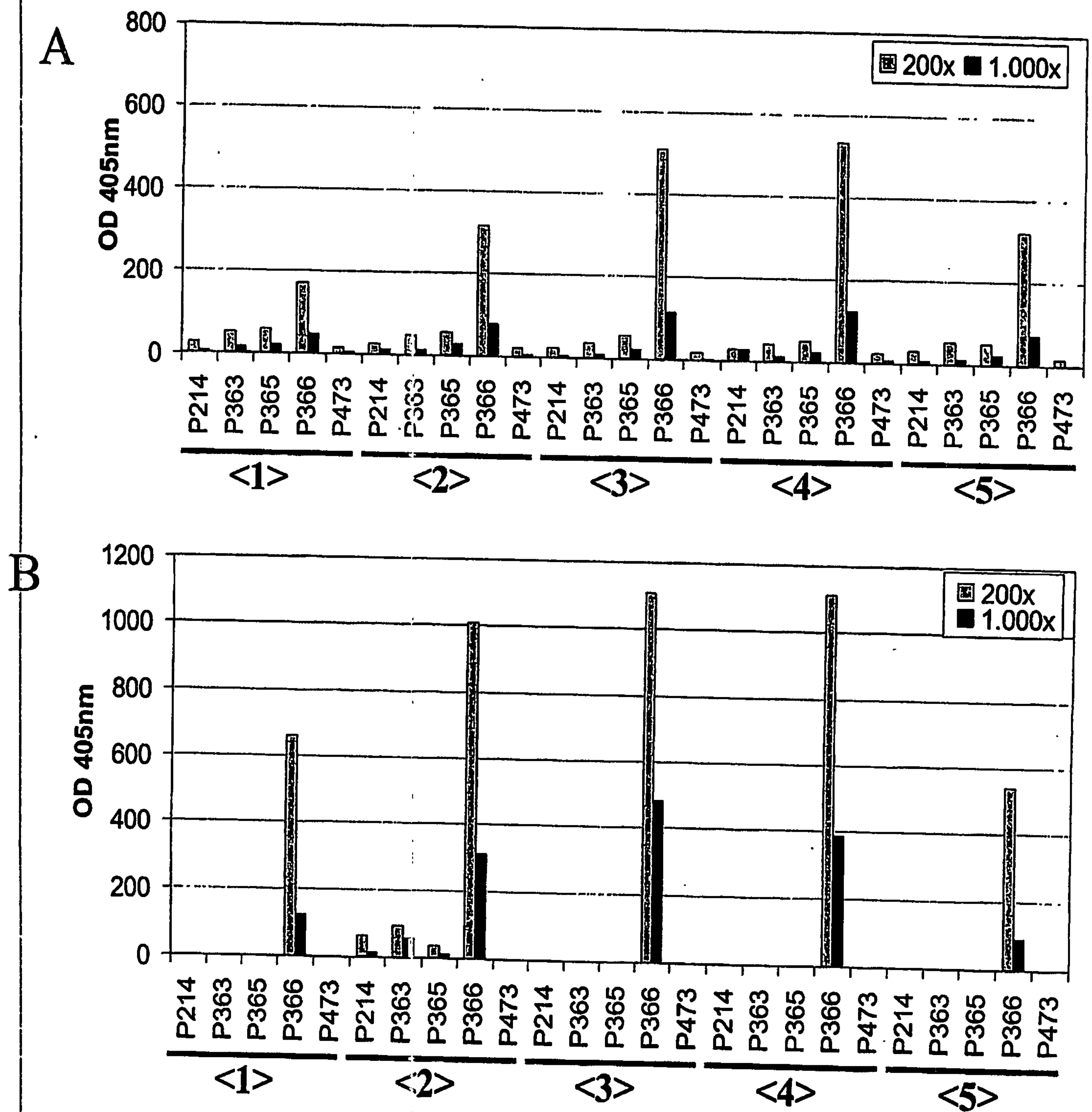

A.
adherence [%]
100
90
80
70
60
50
40
30
20
10
0
6313
6313 ΔfbsA
706 S2
706 S2 ΔfbsA
O90R
O90R ΔfbsA
B.
invasion [%]
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
6313
6313 ΔfbsA
706 S2
706 S2 ΔfbsA
O90R
O90R ΔfbsA

Fig. 14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600
400
200
0
OD 405nm
200x 1.000x
P214 P363 P365 P366 P473 P214 P363 P365 P366 P473 P214 P363 P365 P366 P473 P214 P363 P365 P366 P473 P214 P363 P365 P366 P473
<1> <2> <3> <4> <5>
B
1200
1000
800
600
400
200
0
OD 405nm
200x
1.000x
P214 P363 P365 P366 P473 P214 P363 P365 P366 P473 P214 P363 P365 P366 P473 P214 P363 P365 P366 P473 P214 P363 P365 P366 P473
<1> <2> <3> <4> <5>

Fig. 15

1
ATTTTTAAGCAATATTTTAAAACATAAAAAAAGAAAAATCAACTACTTAAGCTAATTGAA
61
GTATTTCTAAGATAATAAAAAATAAGATTATCAAATAAAAAGAAAAATCATTCAAAAATT
121
GGGAAAAAACTTTAAAATTCCATACCTTATAATAAGAAATTATTGATATCATAATAAGTG
181
ATAGTTTGTATATTCTAGGATATTCTGTATCTGATCTTAGATTTAGAAACGACATTTCGG
241
CACAATAGGAGTTGTAAAATGAGAAAATACCAAAAATTTTCTAAAATATTGACGTTAAGT
301 RBS *M R K Y Q K F S K I L T L S*
CTTTTTTGTTTGTCGCAAATACCGCTTAATACCAATGTTTTAGGGGAAAGTACCGTACCG
L F C L S Q I P L N T N V L G E S T V P
361
GAAAATGGTGCTAAAGGAAAGTTAGTTGTTAAAAAGACAGATGACCAGAACAAACCACTT
E N G A K G K L V V K K T D D Q N K P L
421
TCAAAAGCTACCTTTGTTTTAAAAACTACTGCTCATCCAGAAAGTAAAATAGAAAAAGTA
S K A T F V L K T T A H P E S K I E K V
481
ACTGCTGAGCTAACAGGTGAAGCTACTTTTGATAATCTCATACCTGGAGATTATACTTTA
T A E L T G E A T F D N L I P G D Y T L
541
TCAGAAGAAACAGCGCCCGAAGGTTATAAAAAGACTAACCAGACTTGGCAAGTTAAGGTT
S E E T A P E G Y K K T N Q T W Q V K V
601
GAGAGTAATGGAAAAACTACGATACAAAATAGTGGTGATAAAAATTCCACAATTGGACAA
E S N G K T T I Q N S G D K N S T I G Q
661
AATCACGAAGAACTAGATAAGCAGTATCCCCCCACAGGAATTTATGAAGATACAAAGGAA
N H E E L D K Q Y P P T G I Y E D T K E
721
TCTTATAAACTTGAGCATGTTAAAGGTTCAGTTCCAAATGGAAAGTCAGAGGCAAAAGCA
S Y K L E H V K G S V P N G K S E A K A
781
GTTAACCCATATTCAAGTGAAGGTGAGCATATAAGAGAAATTCCAGAGGGAACATTATCT
V N P Y S S E G E H I R E I P E G T L S
841
AAACGTATTTCAGAAGTAGGTGATTTAGCTCATAATAAATATAAAATTGAGTTAACTGTC
K R I S E V G D L A H N K Y K I E L T V
901
AGTGGAAAAACCATAGTAAAACCAGTGGACAAACAAAAGCCGTTAGATGTTGTCTTCGTA
S G K T I V K P V D K Q K P L D V V F V
961
CTCGATAATTCTAACTCAATGAATAACGATGGCCCAAATTTTCAAAGGCATAATAAAGCC
L D N S N S M N N D G P N F Q R H N K A
1021

Fig. 16-1

AAGAAAGCTGCCGAAGCTCTTGGGACCGCAGTAAAAGATATTTTAGGAGCAAACAGTGAT
K K A A E A L G T A V K D I L G A N S D
1081
AATAGGGTTGCATTAGTTACCTATGGTTCAGATATTTTTGATGGTAGGAGTGTAGATGTC
N R V A L V T Y G S D I F D G R S V D V
1141
GTAAAAGGATTTAAAGAAGATGATAAATATTATGGCCTTCAAACTAAGTTCACAATTCAG
V K G F K E D D K Y Y G L Q T K F T I Q
1201
ACAGAGAATTATAGTCATAAACAATTAACAAATAATGCTGAAGAGATTATAAAAAGGATT
T E N Y S H K Q L T N N A E E I I K R I
1261
CCTACAGAAGCTCCTAGAGCTAAATGGGGATCAACTACAAACGGACTTACTCCAGAGCAA
P T E A P R A K W G S T T N G L T P E Q
1321
CAAAAGCAGTACTATCTTAGTAAAGTAGGGGAAACATTTACTATGAAAGCCTTCATGGAG
Q K Q Y Y L S K V G E T F T M K A F M E
1381
GCAGATGATATTTTGAGTCAAGTAGATCGAAATAGTCAAAAAATTATTGTTCATATAACT
A D D I L S Q V D R N S Q K I I V H I T
1441
GATGGTGTTCCAACAAGATCATATGCTATTAATAATTTTAAATTGGGTGCATCATATGAA
D G V P T R S Y A I N N F K L G A S Y E
1501
AGCCAATTTGAACAAATGAAAAAAAATGGATATCTAAATAAAAGTAATTTTCTACTTACT
S Q F E Q M K K N G Y L N K S N F L L T
1561
GATAAGCCCGAGGATATAAAAGGAAATGGGGAGAGTTACTTTTTGTTTCCCTTAGATAGT
D K P E D I K G N G E S Y F L F P L D S
1621
TATCAAACACAGATAATCTCTGGAAACTTACAAAAACTTCATTATTTAGATTTAAATCTT
Y Q T Q I I S G N L Q K L H Y L D L N L
1681
AATTACCCTAAAGGTACAATTTATCGAAATGGACCAGTAAGAGAACATGGAACACCAACC
N Y P K G T I Y R N G P V R E H G T P T
1741
AAACTTTATATAAATAGTTTAAAACAGAAAAATTATGACATCTTTAATTTTGGTATAGAT
K L Y I N S L K Q K N Y D I F N F G I D
1801
ATATCTGCTTTTAGACAAGTTTATAATGAGGATTATAAGAAAAATCAAGATGGTACTTTT
I S A F R Q V Y N E D Y K K N Q D G T F
1861
CAAAAATTGAAAGAGGAAGCTTTTGAACTTTCAGATGGGGAAATAACAGAACTAATGAAG
Q K L K E E A F E L S D G E I T E L M K
1921
TCATTCTCTTCTAAACCTGAGTATTATACCCCGATAGTAACTTCATCCGATGCATCTAAC
S F S S K P E Y Y T P I V T S S D A S N
1981

Fig. 16-2

AATGAAATTTTATCTAAAATTCAGCAACAATTTGAAAAGGTTTTAACAAAAGAAAACTCA
N E I L S K I Q Q Q F E K V L T K E N S
2041
ATTGTTAATGGAACTATAGAAGATCCTATGGGTGACAAAATCAATTTACAGCTTGGCAAC
I V N G T I E D P M G D K I N L Q L G N
2101
GGACAAACATTGCAACCAAGTGATTATACTTTACAGGGAAATGATGGAAGTATAATGAAA
G Q T L Q P S D Y T L Q G N D G S I M K
2161
GATAGCATTGCAACTGGTGGGCCTAATAATGATGGTGGAATACTTAAAGGGGTTAAATTA
D S I A T G G P N N D G G I L K G V K L
2221
GAATACATCAAAAATAAACTCTACGTTAGAGGTTTGAACTTAGGGGAGGGACAAAAAGTA
E Y I K N K L Y V R G L N L G E G Q K V
2281
ACACTCACATATGATGTGAAACTAGATGACAGTTTTATAAGTAACAAATTCTATGACACT
T L T Y D V K L D D S F I S N K F Y D T
2341
AATGGTAGAACAACATTGAATCCTAAATCAGAGGATCCTAATACACTTAGAGATTTTCCA
N G R T T L N P K S E D P N T L R D F P
2401
ATCCCTAAAATTCGTGATGTGAGAGAATATCCTACAATAACGATTAAAAACGAGAAGAAG
I P K I R D V R E Y P T I T I K N E K K
2461
TTAGGTGAAATTGAATTTACAAAAGTTGATAAAGATAATAATAAGTTGCTTCTCAAAGGA
L G E I E F T K V D K D N N K L L L K G
2521
GCTACGTTTGAACTTCAAGAATTTAATGAAGATTATAAACTTTATTTACCAATAAAAAAT
A T F E L Q E F N E D Y K L Y L P I K N
2581
AATAATTCAAAAGTAGTGACGGGAGAAAACGGCAAAATTTCTTACAAAGATTTGAAAGAT
N N S K V V T G E N G K I S Y K D L K D
2641
GGCAAATATCAGTTAATAGAAGCAGTTTCGCCGAAGGATTATCAAAAAATTACTAATAAA
G K Y Q L I E A V S P K D Y Q K I T N K
2701
CCAATTTTAACTTTTGAAGTTGTTAAAGGATCGATACAAAATATAATAGCTGTTAATAAA
P I L T F E V V K G S I Q N I I A V N K
2761
CAGATTTCTGAATATCATGAGGAAGGTGACAAGCATTTAATTACCAACACGCATATTCCA
Q I S E Y H E E G D K H L I T N T H I P
2821
CCAAAAGGAATTATTCCGATGACAGGTGGGAAAGGAATTCTATCTTTCATTTTAATAGGT
P K G I I P M T G G K G I L S F I L I G
2881
GGATCTATGATGTCTATTGCAGGTGGAATTTATATTTGGAAAAGATATAAGAAATCTAGT
G S M M S I A G G I Y I W K R Y K K S S
2941

Fig. 16-3

GATATATCTAGAGAAAAAGATTAAGAATCATGTGTTTTAGTATTCTTAATTAATTAAATA
D I S R E K D *
3001
TAATTCGAAAGGAGTGGTGCTGCGGTAATATTATAATCCGTATATTATTATCTATGTTGA
3061
TTAACTAGAATAAGAAGGAGATAGAAATGAAAAAAATCAACAAATGTCTTACAGTGTTCT
3121 RBS *M K K I N K C L T V F*
CGACACTGCTATTGATCTTAACGTCACTATTCTCAGTTGCACCAGCGTTTGCGGACGACG
S T L L L I L T S L F S V A P A F A D D
3181
TAACAACTGATACTGTGACCTTGCACAAGATTGTCATGCCACAAGCTGCATTTGATAACT
V T T D T V T L H K I V M P Q A A F D N
3241
TTACTGAAGGTACAAAAGGTAAGAATGATAGCGATTATGTTGGTAAACAAATTAATGACC
F T E G T K G K N D S D Y V G K Q I N D
3301
TTAAATCTTATTTTGGCTCAACCGATGCTAAAGAAATTAAGGGTGCTTTCTTTGTTTTCA
L K S Y F G S T D A K E I K G A F F V F
3361
AAAATGAAACTGGTACAAAATTCATTACTGAAAATGGTAAGGAAGTCGATACTTTGGAAG
K N E T G T K F I T E N G K E V D T L E
3421
CTAAAGATGCTGAAGGTGGTGCTGTTCTTTCAGGGTTAACAAAAGACACTGGTTTTGCTT
A K D A E G G A V L S G L T K D T G F A
3481
TTAACACTGCTAAGTTAAAAGGAACTTACCAAATCGTTGAATTGAAAGAAAAATCAAACT
F N T A K L K G T Y Q I V E L K E K S N
3541
ACGATAACAACGGTTCTATCTTGGCTGATTCAAAAGCAGTTCCAGTTAAAATCACTCTGC
Y D N N G S I L A D S K A V P V K I T L
3601
CATTGGTAAACAACCAAGGTGTTGTTAAAGATGCTCACATTTATCCAAAGAATACTGAAA
P L V N N Q G V V K D A H I Y P K N T E
3661
CAAAACCACAAGTAGATAAGAACTTTGCAGATAAAGATCTTGATTATACTGACAACCGAA
T K P Q V D K N F A D K D L D Y T D N R
3721
AAGACAAAGGTGTTGTCTCAGCGACAGTTGGTGACAAAAAAGAATACATAGTTGGAACAA
K D K G V V S A T V G D K K E Y I V G T
3781
AAATTCTTAAAGGCTCAGACTATAAGAAACTGGTTTGGACTGATAGCATGACTAAAGGTT
K I L K G S D Y K K L V W T D S M T K G
3841
TGACGTTCAACAACAACGTTAAAGTAACATTGGATGGTAAAGATTTTCCTGTTTTAAACT
L T F N N N V K V T L D G K D F P V L N
3901
ACAAACTCGTAACAGATGACCAAGGTTTCCGTCTTGCCTTGAATGCAACAGGTCTTGCAG
Y K L V T D D Q G F R L A L N A T G L A
3961

Fig. 16-4

CAGTAGCAGCTGCTGCAAAAGACAAAGATGTTGAAATCAAGATCACTTACTCAGCTACGG
A V A A A A K D K D V E I K I T Y S A T
4021
TGAACGGCTCCACTACTGTTGAAGTTCCAGAAACCAATGATGTTAAATTGGACTATGGTA
V N G S T T V E V P E T N D V K L D Y G
4081
ATAACCCAACGGAAGAAAGTGAACCACAAGAAGGTACTCCAGCTAACCAAGAAATTAAAG
N N P T E E S E P Q E G T P A N Q E I K
4141
TCATTAAAGACTGGGCAGTAGATGGTACAATTACTGATGTTAATGTTGCAGTTAAAGCTA
V I K D W A V D G T I T D V N V A V K A
4201
TCTTTACCTTGCAAGAAAAACAAACGGATGGTACATGGGTGAACGTTGCTTCACACGAAG
I F T L Q E K Q T D G T W V N V A S H E
4261
CAACAAAACCATCACGCTTTGAACATACTTTCACAGGTTTGGATAATACTAAAACTTACC
A T K P S R F E H T F T G L D N T K T Y
4321
GCGTTGTCGAACGTGTTAGCGGCTACACTCCAGAATATGTATCATTTAAAAATGGTGTTG
R V V E R V S G Y T P E Y V S F K N G V
4381
TGACTATCAAGAACAACAAAAACTCAAATGATCCAACTCCAATCAACCCATCAGAACCAA
V T I K N N K N S N D P T P I N P S E P
4441
AAGTGGTGACTTATGGACGTAAATTTGTGAAAACAAATCAAGCTAACACTGAACGCTTGG
K V V T Y G R K F V K T N Q A N T E R L
4501
CAGGAGCTACCTTCCTTGTTAAGAAAGAAGGAAAATACTTGGCACGTAAAGCAGGTGCAG
A G A T F L V K K E G K Y L A R K A G A
4561
CAACTGCTGAAGCAAAGGCAGCTGTAAAAACTGCTAAACTAGCATTGGATGAAGCTGTTA
A T A E A K A A V K T A K L A L D E A V
4621
AAGCTTATAACGACTTGACTAAAGAAAAACAAGAAGGCCAAGAAGGTAAAACAGCATTGG
K A Y N D L T K E K Q E G Q E G K T A L
4681
CTACTGTTGATCAAAAACAAAAAGCTTACAATGACGCTTTTGTTAAAGCTAACTACTCAT
A T V D Q K Q K A Y N D A F V K A N Y S
4741
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Y E W V A D K K A D N V V K L I S N A G
4801
GTCAATTTGAAATTACTGGTTTGGATAAAGGCACTTATAGCTTGGAAGAAACTCAAGCAC
G Q F E I T G L D K G T Y S L E E T Q A
4861
CAGCAGGTTATGCGACATTGTCAGGTGATGTAAACTTTGAAGTAACTGCCACATCATATA
P A G Y A T L S G D V N F E V T A T S Y
4921

Fig. 16-5

GCAAAGGGGCTACAACTGACATCGCATATGATAAAGGATCTGTAAAAAAAGATGCCCAAC
S K G A T T D I A Y D K G S V K K D A Q
4981
AAGTTCAAAACAAAAAAGTAACCATCCCACAAACAGGTGGTATTGGTACAATTCTTTTCA
Q V Q N K K V T I P Q T G G I G T I L F
5041
CAATTATTGGTTTAAGCATTATGCTTGGAGCAGTAGTTGTCATGAAAAAACGTCAATCAG
T I I G L S I M L G A V V V M K K R Q S
5101
AGGAAGCTTAAGGCTAGTCTTTGATGGTGTATAAGCACAGTTAAAGCTGTGCTTATGATC
E E A *
5161
TAAGGGTATTTCAGTAGAAGTACTCTTAGATCATAAGCAAGAGCCATTATTTAGGAGATG
5221
ACGTGAAGACTAAAAATATCAACAAAAAAACTAAAAAGAAGAAGTCAAATCTTCCTTTTA
5281
TCATTCTTTTTCTAATAGGTCTATCTATTTTATTGTATCCAGTGGTATCACGTTTTTACT
5341
ATACGATAGAATCTAATAATCAAACACAGGATTTTGAGAGAG

Fig. 16-6

1
GCTCATGATAATTTATAGAACATTTATAAAATCTTATAATAAACTGGTTAAGTATAGGAA
61
ATACTGCATATTTCTTGAAAATATGGTGTATATTGTGAATAAAATGATGACCAAGTTAAT
121
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181
GTCAAATAAATAGAGCCTTTATAAAAATATTATATAAGTATAAAATGTAAAAAAATAAAA
241
AAATGATATTTTTATTTGATTCAAATGTATTTAATAAAAATACAAAGTTTCTAAAAAAGT
301
AAAAATTCCATCTCAATAAACAGCGTTAGTTATTATAACCGAACATTATTGTCCTTAAAA
361
CATTAAAACAAAAACAAAAGTTCGTAATTTAATTAATTTGTCATGTTACTAATCTTATGC
421
TAATATATTATCTCGTGATAAGTTTTTGATGTAAAAATTATCATGAAAAAGAAAAGAGAG
481 RBS
ATGGAAATGAAAAAACAATTTTTAAAATCAGCAGCGATTCTATCGCTAGCAGTAACAGCA
541 ***M K K Q F L K S A A I L S L A V T A***
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V S T S Q P V A G I T K D Y N N R N E K
601
GTAAAAAAGTATTTACAAGAAAATAATTTCGGTCATAAAATAGCGTATGGATGGAAAAAT
V K K Y L Q E N N F G H K I A Y G W K N
661
AAAGTAGAATTTGATTTTCGTTATTTATTGGATACTGCTAAATATTTAGTAAATAAAGAA
K V E F D F R Y L L D T A K Y L V N K E
721
GAATTTCAAGATCCTTTATATAATGATGCGCGCGAAGAATTGATAAGTTTTATTTTTCCT
E F Q D P L Y N D A R E E L I S F I F P
781
TATGAGAAATTTTTAATTAACAATCGTGACATAACTAAATTAACAGTTAATCAGTATGAA
Y E K F L I N N R D I T K L T V N Q Y E
841
GCGATTGTGAATAGAATGAGTGTTGCTTTACAAAAATTTTCAAAGAATATTTTTGAGAAA
A I V N R M S V A L Q K F S K N I F E K
901
CAGAAAGTAAATAAAGATTTAATCCCTATTGCGTTTTGGATTGAGAAAAGTTACAGAACT
Q K V N K D L I P I A F W I E K S Y R T
961
GTTGGAACGAATGAAATCGCCGCTTCTGTAGGCATTCAAGGAGGATTTTATCAAAACTTC
V G T N E I A A S V G I Q G G F Y Q N F
1021
CATGATTATTATAATTATTCATATCTATTAAATTCTTTATGGCATGAAGGAAATGTAAAA
H D Y Y N Y S Y L L N S L W H E G N V K
1081
GAAGTAGTTAAGGATTATGAAAACACTATTCGTCAAATACTATCTAAAAAGCATGAGATT
E V V K D Y E N T I R Q I L S K K H E I
1141

Fig. 17-1

GAAAAAATTCTTAATCAGAGCACTTCTGATATCTCTATAGATGATGATGATTACGAAAAA
E K I L N Q S T S D I S I D D D D Y E K
1201
GGAAATAAAGAATTGCTAAGGGAAAAATTAAATATTATTCTAAATCTTTCAAAGAGAGAT
G N K E L L R E K L N I I L N L S K R D
1261
TACAGAGTAACTCCATACTATGAAGTGAATAAACTACATACAGGGCTTATTTTATTGGAG
Y R V T P Y Y E V N K L H T G L I L L E
1321
GATGTCCCTAATTTAAAGATTGCTAAGGATAAGTTGTTCTCATTAGAGAATTCTTTAAAG
D V P N L K I A K D K L F S L E N S L K
1381
GAATACAAAGGAGAGAAAGTTAATTATGAGGAACTAAGATTCAATACGGAACCTTTAACT
E Y K G E K V N Y E E L R F N T E P L T
1441
AGTTACTTAGAAAATAAAGAAAAATTTTTAGTCCCCAATATTCCATATAAAAATAAATTA
S Y L E N K E K F L V P N I P Y K N K L
1501
ATTTTAAGGGAAGAAGATAAATATAGTTTTGAAGATGATGAAGAAGAGTTTGGAAATGAA
I L R E E D K Y S F E D D E E E F G N E
1561
CTTCTAAGTTACAATAAGCTTAAGAATGAAGTTTTACCTGTTAATATTACAACTTCTACT
L L S Y N K L K N E V L P V N I T T S T
1621
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I L K P F E Q K K I V E D F N P Y S N L
1681
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D N L E I K K I R L N G S Q K Q K V E Q
1741
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E K T K S P T P Q K E T V K E Q T E Q K
1801
GTATCTGGAAATACTCAAGAGGTAGAAAAGAAATCTGAAACTGTGGCAACTTCACAACAA
V S G N T Q E V E K K S E T V A T S Q Q
1861
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S S V A Q T S V Q Q P A P V Q S V V Q E
1921
TCCAAAGCTTCTCAAGAGGAGATTAATGCAGCACACGATGCTATTTCGGCGTATAAATCA
S K A S Q E E I N A A H D A I S A Y K S
1981
ACAGTCAATATTGCTAATACAGCCGGTGTAACAACTGCGGAAATGACCACGCTCATTAAT
T V N I A N T A G V T T A E M T T L I N
2041
ACTCAAACTTCTAATCTTTCTGATGTTGAGAAAGCTTTAGGAAATAATAAGGTTAATAAT
T Q T S N L S D V E K A L G N N K V N N
2101
GGTGCAGTCAATGTATTGAGAGAAGATACAGCTCGTCTTGAGAATATGATTTGGAATCGT
G A V N V L R E D T A R L E N M I W N R
2161

Fig. 17-2

GCTTACCAAGCTATTGAAGAATTCAACGTCGCTCGTAATACTTATAATAACCAAATCAAG
A Y Q A I E E F N V A R N T Y N N Q I K
2221
ACAGAAACAGTTCCAGTTGATAATGATATTGAAGCTATTTTAGCAGGTTCTCAAGCTAAA
T E T V P V D N D I E A I L A G S Q A K
2281
ATTAGCCATTTGGACAATCGTATCGGAGCGCGCCACATGGATCAAGCTTTTGTAGCTAGT
I S H L D N R I G A R H M D Q A F V A S
2341
TTATTAGAAGTTACTGAGATGAGTAAATCAATCTCATCGCGTATAAAAGAGTAGACACTG
L L E V T E M S K S I S S R I K E *
2401
CTATCAAGGCGATCTTAAACTTTTGTATTAAACTAACCTAAAAGATAGAAAGAGACTAAT
2461 RBS
ATGAAAAAAATAACAACTTTAATCTTAGCTAGTAGCTTATTACTAGTTGCAACGACATCG
M K K I T T L I L A S S L L L V A T T S
2521
GTTAAAGCTGATGATAACTTTGAAATGCCAACGCGTTATGTTAAAATGAGTGAAAAATCA
V K A D D N F E M P T R Y V K M S E K S
2581
AAAGCATTTTATCAAAGACTACAAGAAAAACAACGTAAGGCACATACTACTGTGAAGACT
K A F Y Q R L Q E K Q R K A H T T V K T
2641
TTTAATAATTCAGAAATAAGGCATCAACTACCTCTTAAACAAGAAAAGGCTAGAAATGAT
F N N S E I R H Q L P L K Q E K A R N D
2701
ATCTACAATTTAGGCATTCTTATTTCTCAGGAGTCTAAAGGGTTCATCCAACGTATTGAT
I Y N L G I L I S Q E S K G F I Q R I D
2761
AATGCCTATTCTTTGGAAAATGTCTCAGATATTGTTAATGAAGCTCAGGCTTTGTATAAA
N A Y S L E N V S D I V N E A Q A L Y K
2821
CGTAACTATGATTTATTTGAAAAAATCAAATCTACACGTGATAAGGTTCAAGTCTTACTT
R N Y D L F E K I K S T R D K V Q V L L
2881
GCATCGCATCAAGATAATACAGACTTAAAAAACTTTTATGCTGAGTTAGATGATATGTAT
A S H Q D N T D L K N F Y A E L D D M Y
2941
GAACATGTTTATCTCAATGAAAGTAGAGTGGAGGCGATAAACAGAAATATCCAAAAATAT
E H V Y L N E S R V E A I N R N I Q K Y
3001
AATTAGTTTCTAAACTAACAAACATTCCTAAATATAAGATATTAAACCCTACTTATTGAT
N *
3061
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GCTGAGATTATACCCATTGA

Fig. 17-3

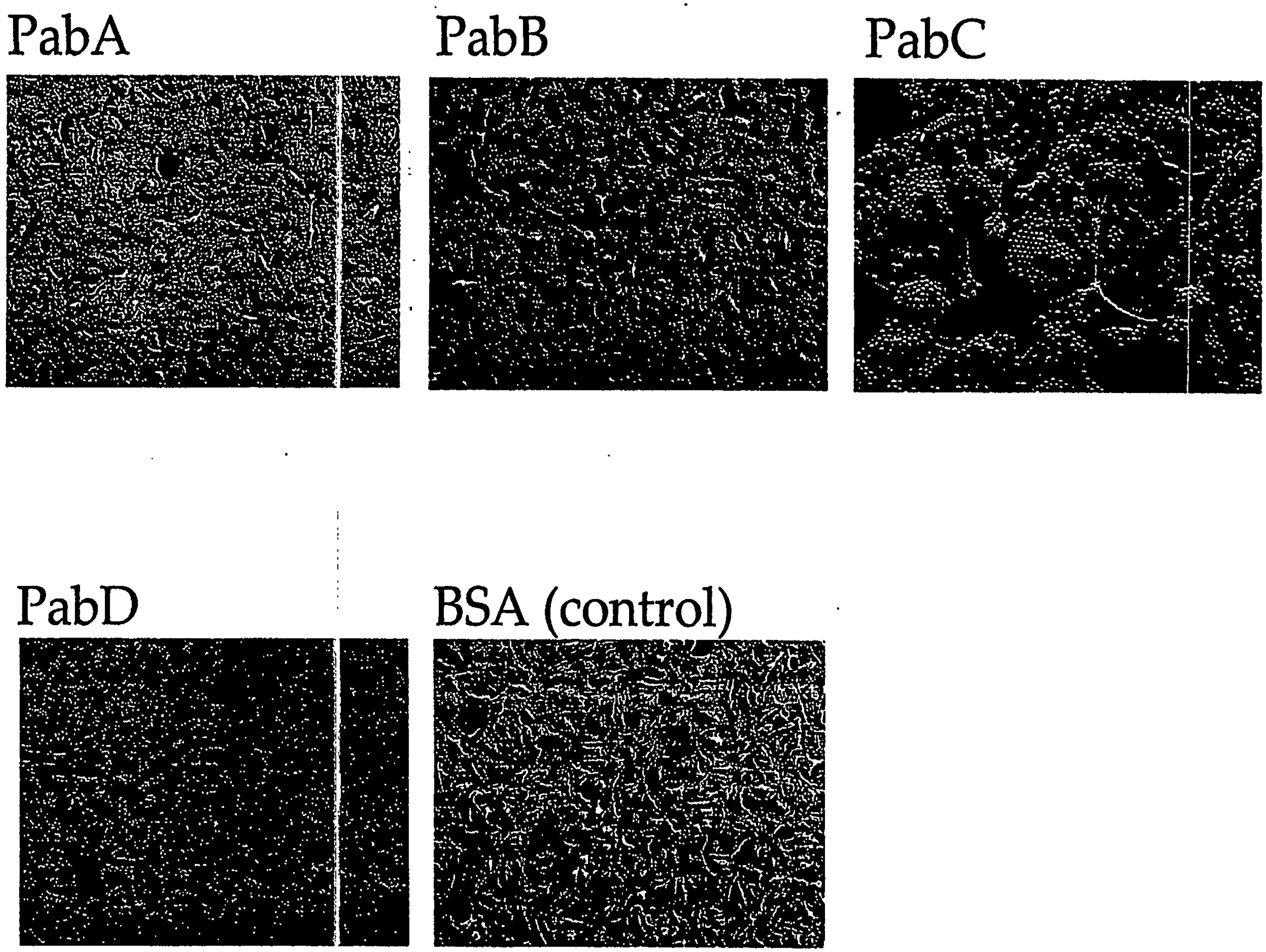
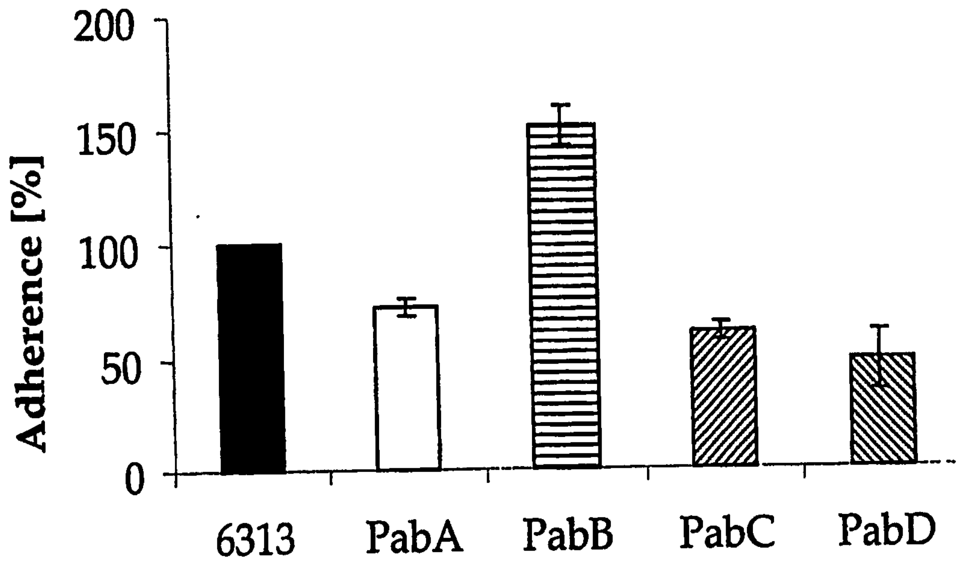


Fig. 18

A.

Adherence of GBS 6313 in the presence of 100 µg/ml of different fusion proteins



B.

Invasion of GBS 6313 in the presence of 100µg/ml of different fusion proteins

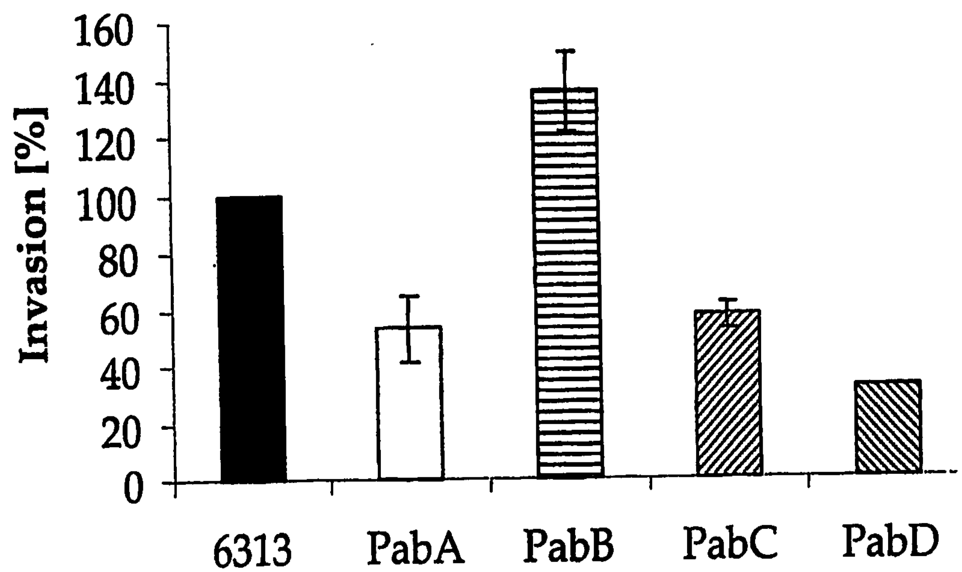
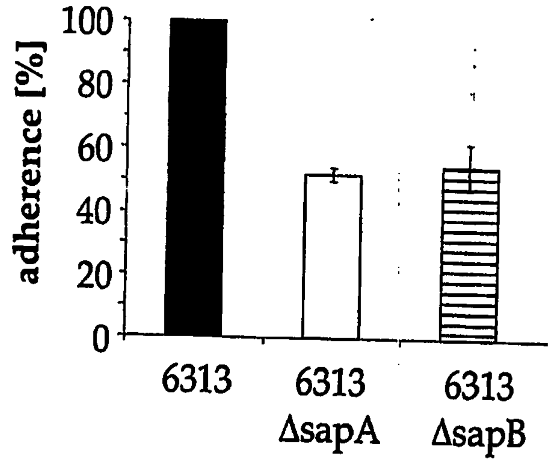


Fig. 19

A.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60
40
20
0
6313
6313 ΔsapA
6313 ΔsapB

B.

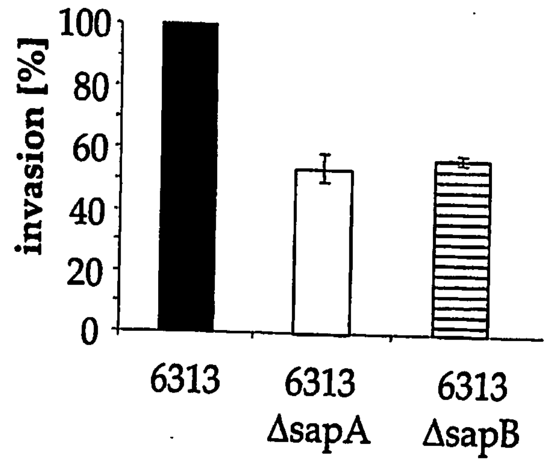
invasion [%]
100
80
60
40
20
0
6313
6313 ΔsapA
6313 ΔsapB

Fig. 20

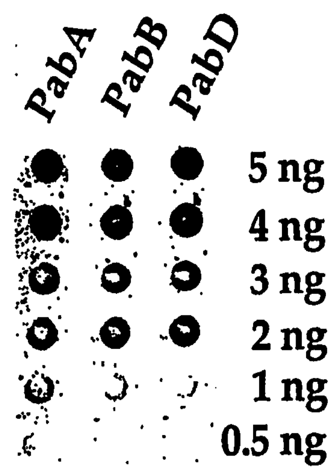

Fig. 21

PO - Munich
3
15. Okt. 2002

SEQUENCE LISTING

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aatcaaagtc agggcaatgt tttagagcgt cgtcaacgcg atgcagaaaa cagaagccaa 180

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gagcgtcgtc aacgtgatgc ggaaaacaag agccaagtag gtcaacttat agggaaaaat 300

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<210> 6
<211> 1857
<212> DNA
<213> Streptococcus agalactiae

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gagcgtcgtc aacgtgatgc agataacaaa agtcagggca atgttttaga gcgtcgtcaa 1260

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aacaagagcc agggcaatgt tctagaacgt cgtcaacgtg atgcggataa caagagccaa 1380

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cgcgatgcag ataacaagag ccagggtaat gttctagagc gtcgccaacg cgatgcggaa 1560

aacaaaagtc aagtaggtca acttataggg aaaaatccac ttttttcaaa gtcaactgta 1620

tctagagaaa ataatcactc tagtcaaggt gactctaaca aacagtcatt ctctaaaaaa 1680

atatctcagg ttactaatgt agctaatgga ccgatgttaa ctaataattc tagaacaatt 1740

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<210> 7
<211> 2706
<212> DNA
<213> Streptococcus agalactiae

<400> 7
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aagttagttg ttaaaaagac agatgaccag aacaaaccac tttcaaaagc tacctttgtt 180

ttaaaaacta ctgctcatcc agaaagtaaa atagaaaaag taactgctga gctaacaggt 240

gaagctactt ttgataatct catacctgga gattatactt tatcagaaga aacagcgccc 300

gaaggttata aaaagactaa ccagacttgg caagttaagg ttgagagtaa tggaaaaact 360

acgatacaaa atagtggtga taaaaattcc acaattggac aaaatcacga agaactagat 420

aagcagtatc cccccacagg aatttatgaa gatacaaagg aatcttataa acttgagcat 480

gttaaaggtt cagttccaaa tggaaagtca gaggcaaaag cagttaaccc atattcaagt 540

gaaggtgagc atataagaga aattccagag ggaacattat ctaaacgtat ttcagaagta 600

ggtgatttag ctcataataa atataaaatt gagttaactg tcagtggaaa aaccatagta 660

aaaccagtgg acaaacaaaa gccgttagat gttgtcttcg tactcgataa ttctaactca 720

atgaataacg atggcccaaa ttttcaaagg cataataaag ccaagaaagc tgccgaagct 780

cttgggaccg cagtaaaaga tattttagga gcaaacagtg ataatagggt tgcattagtt 840

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gatgataaat attatggcct tcaaactaag ttcacaattc agacagagaa ttatagtcat 960

aaacaattaa caaataatgc tgaagagatt ataaaaagga ttcctacaga agctcctaga 1020

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caagtagatc gaaatagtca aaaaattatt gttcatataa ctgatggtgt tccaacaaga 1200

tcatatgcta ttaataattt taaattgggt gcatcatatg aaagccaatt tgaacaaatg 1260

aaaaaaaatg gatatctaaa taaaagtaat tttctactta ctgataagcc cgaggatata 1320

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tctggaaact tacaaaaact tcattattta gatttaaatc ttaattaccc taaaggtaca 1440

atttatcgaa atggaccagt aagagaacat ggaacaccaa ccaaacttta tataaatagt 1500

ttaaaacaga aaaattatga catctttaat tttggtatag atatatctgc ttttagacaa 1560

gtttataatg aggattataa gaaaaatcaa gatggtactt ttcaaaaatt gaaagaggaa 1620

gcttttgaac tttcagatgg ggaaataaca gaactaatga agtcattctc ttctaaacct 1680

gagtattata ccccgatagt aacttcatcc gatgcatcta acaatgaaat tttatctaaa 1740

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acaaaagttg ataaagataa taataagttg cttctcaaag gagctacgtt tgaacttcaa 2280

gaatttaatg aagattataa actttattta ccaataaaaa ataataattc aaaagtagtg 2340

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gaagcagttt cgccgaagga ttatcaaaaa attactaata aaccaatttt aacttttgaa 2460

gttgttaaag gatcgataca aaatataata gctgttaata aacagatttc tgaatatcat 2520

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<211> 2025
<212> DNA
<213> Streptococcus agalactiae

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aagattgtca tgccacaagc tgcatttgat aactttactg aaggtacaaa aggtaagaat 180

gatagcgatt atgttggtaa acaaattaat gaccttaaat cttattttgg ctcaaccgat 240

gctaaagaaa ttaagggtgc tttctttgtt ttcaaaaatg aaactggtac aaaattcatt 300

actgaaaatg gtaaggaagt cgatactttg gaagctaaag atgctgaagg tggtgctgtt 360

ctttcagggt taacaaaaga cactggtttt gcttttaaca ctgctaagtt aaaaggaact 420

taccaaatcg ttgaattgaa agaaaaatca aactacgata acaacggttc tatcttggct 480

gattcaaaag cagttccagt taaaatcact ctgccattgg taaacaacca aggtgttgtt 540

aaagatgctc acatttatcc aaagaatact gaaacaaaac cacaagtaga taagaacttt 600

gcagataaag atcttgatta tactgacaac cgaaaagaca aaggtgttgt ctcagcgaca 660

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aaactggttt ggactgatag catgactaaa ggtttgacgt tcaacaacaa cgttaaagta 780

acattggatg gtaaagattt tcctgtttta aactacaaac tcgtaacaga tgaccaaggt 840

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gatgttgaaa tcaagatcac ttactcagct acggtgaacg gctccactac tgttgaagtt 960

ccagaaacca atgatgttaa attggactat ggtaataacc caacggaaga aagtgaacca 1020

caagaaggta ctccagctaa ccaagaaatt aaagtcatta aagactgggc agtagatggt 1080

acaattactg atgttaatgt tgcagttaaa gctatcttta ccttgcaaga aaaacaaacg 1140

gatggtacat gggtgaacgt tgcttcacac gaagcaacaa aaccatcacg ctttgaacat 1200

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<210> 9
<211> 1908
<212> DNA
<213> Streptococcus agalactiae

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gaatttgatt ttcgttattt attggatact gctaaatatt tagtaaataa agaagaattt 240

caagatcctt tatataatga tgcgcgcgaa gaattgataa gttttatttt tccttatgag 300

aaatttttaa ttaacaatcg tgacataact aaattaacag ttaatcagta tgaagcgatt 360

gtgaatagaa tgagtgttgc tttacaaaaa ttttcaaaga atatttttga gaaacagaaa 420

gtaaataaag atttaatccc tattgcgttt tggattgaga aaagttacag aactgttgga 480

acgaatgaaa tcgccgcttc tgtaggcatt caaggaggat tttatcaaaa cttccatgat 540

tattataatt attcatatct attaaattct ttatggcatg aaggaaatgt aaaagaagta 600

gttaaggatt atgaaaacac tattcgtcaa atactatcta aaaagcatga gattgaaaaa 660

attcttaatc agagcacttc tgatatctct atagatgatg atgattacga aaaaggaaat 720

aaagaattgc taagggaaaa attaaatatt attctaaatc tttcaaagag agattacaga 780

gtaactccat actatgaagt gaataaacta catacagggc ttattttatt ggaggatgtc 840

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agggaagaag ataaatatag ttttgaagat gatgaagaag agtttggaaa tgaacttcta 1080

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caagctattg aagaattcaa cgtcgctcgt aatacttata ataaccaaat caagacagaa 1740

acagttccag ttgataatga tattgaagct attttagcag gttctcaagc taaaattagc 1800

catttggaca atcgtatcgg agcgcgccac atggatcaag cttttgtagc tagtttatta 1860

gaagttactg agatgagtaa atcaatctca tcgcgtataa aagagtag 1908

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<211> 546
<212> DNA
<213> Streptococcus agalactiae

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aaagcatttt atcaaagact acaagaaaaa caacgtaagg cacatactac tgtgaagact 180

tttaataatt cagaaataag gcatcaacta cctcttaaac aagaaaaggc tagaaatgat 240

atctacaatt taggcattct tatttctcag gagtctaaag ggttcatcca acgtattgat 300

aatgcctatt ctttggaaaa tgtctcagat attgttaatg aagctcaggc tttgtataaa 360

cgtaactatg atttatttga aaaaatcaaa tctacacgtg ataaggttca agtcttactt 420

gcatcgcatc aagataatac agacttaaaa aacttttatg ctgagttaga tgatatgtat 480

gaacatgttt atctcaatga aagtagagtg gaggcgataa acagaaatat ccaaaaatat 540

aattag 546

<210> 11
<211> 442
<212> PRT
<213> Streptococcus agalactiae

<400> 11

Met Phe Asn Lys Ile Gly Phe Arg Thr Trp Lys Ser Gly Lys Leu Trp
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20 25 30

Val Ser Ala Met Asp Ser Val Gly Asn Gln Ser Gln Gly Asn Val Leu
35 40 45

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
50 55 60

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
65 70 75 80

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Gly Asn Val Leu
85 90 95

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
100 105 110

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
115 120 125

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
130 135 140

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
145 150 155 160

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
165 170 175

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
180 185 190

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Gly Asn Val Leu
195 200 205

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
210 215 220

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
225 230 235 240

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
245 250 255

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
260 265 270

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
275 280 285

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
290 295 300

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
305 310 315 320

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
325 330 335

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Val Gly Gln Leu
340 345 350

Ile Gly Lys Asn Pro Leu Leu Ser Lys Ser Ile Ile Ser Arg Glu Asn
355 360 365

Asn His Ser Ser Gln Gly Asp Ser Asn Lys Gln Ser Phe Ser Lys Lys
370 375 380

Val Ser Gln Val Thr Asn Val Ala Asn Arg Pro Met Leu Thr Asn Asn
385 390 395 400

Ser Arg Thr Ile Ser Val Ile Asn Lys Leu Pro Lys Thr Gly Asp Asp
405 410 415

Gln Asn Val Ile Phe Lys Leu Val Gly Phe Gly Leu Ile Leu Leu Thr
420 425 430

Ser Arg Cys Gly Leu Arg Arg Asn Glu Asn
435 440

<210> 12
<211> 410
<212> PRT
<213> Streptococcus agalactiae

<400> 12

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Leu Tyr Met Gly Val Leu Gly Ser Thr Ile Ile Leu Gly Ser Ser Ser
20 25 30

Val Ser Ala Met Asp Ser Val Gly Asn Gln Ser Gln Gly Asn Val Leu
35 40 45

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
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Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
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Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
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Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
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Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
115 120 125

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
130 135 140

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
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Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
165 170 175

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
180 185 190

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Gly Asn Val Leu
195 200 205

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
210 215 220

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
225 230 235 240

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
245 250 255

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Gly Asn Val Leu
260 265 270

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
275 280 285

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
290 295 300

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Val Gly Gln Leu
305 310 315 320

Ile Gly Lys Asn Pro Leu Leu Ser Lys Ser Ile Ile Ser Arg Glu Asn
325 330 335

Asn His Ser Ser Gln Gly Asp Ser Asn Lys Gln Ser Phe Ser Lys Lys
340 345 350

Val Ser Gln Val Thr Asn Val Ala Asn Arg Pro Met Leu Thr Asn Asn
355 360 365

Ser Arg Thr Ile Ser Val Ile Asn Lys Leu Pro Lys Thr Gly Asp Asp
370 375 380

Gln Asn Val Ile Phe Lys Leu Val Gly Phe Gly Leu Ile Leu Leu Thr
385 390 395 400

Ser Arg Cys Gly Leu Arg Arg Asn Glu Asn
405 410

<210> 13
<211> 346
<212> PRT
<213> Streptococcus agalactiae

<400> 13

Met Phe Asn Lys Ile Gly Phe Arg Thr Trp Lys Ser Gly Lys Leu Trp
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Leu Tyr Met Gly Val Leu Gly Ser Thr Ile Ile Leu Gly Ser Ser Pro
20 25 30

Val Ser Ala Met Asp Ser Val Gly Asn Gln Ser Gln Gly Asn Val Leu
35 40 45

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
50 55 60

Glu Arg Arg Gln Arg Asp Val Glu Asn Arg Ser Gln Gly Asn Val Leu
65 70 75 80

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
85 90 95

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Gly Asn Val Leu
100 105 110

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
115 120 125

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Gly Asn Val Leu
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Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
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Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
165 170 175

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
180 185 190

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Gly Asn Val Leu
195 200 205

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
210 215 220

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
225 230 235 240

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Val Gly Gln Leu
245 250 255

Ile Gly Lys Asn Pro Leu Leu Ser Lys Ser Thr Ile Ser Arg Glu Asn
260 265 270

Asn His Ser Ser Gln Gly Asp Ser Asn Lys Gln Ser Phe Ser Lys Lys
275 280 285

Val Ser Gln Val Thr Asn Val Ala Asn Arg Pro Met Leu Thr Asn Asn
290 295 300

Ser Arg Thr Ile Ser Val Ile Asn Lys Leu Pro Lys Thr Gly Asp Asp
305 310 315 320

Gln Asn Val Ile Phe Lys Leu Val Gly Phe Gly Leu Ile Leu Leu Thr
325 330 335

Ser Arg Cys Gly Leu Arg Arg Asn Glu Asn
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<210> 14
<211> 186
<212> PRT
<213> Streptococcus agalactiae

<400> 14

Met Phe Asn Lys Ile Gly Phe Arg Thr Trp Lys Ser Gly Lys Leu Trp
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Leu Tyr Met Gly Val Leu Gly Ser Thr Ile Ile Leu Gly Ser Ser Pro
20 25 30

Val Ser Ala Met Asp Ser Val Gly Asn Gln Ser Gln Gly Asn Val Leu
35 40 45

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
50 55 60

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
65 70 75 80

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Val Gly Gln Leu
85 90 95

Ile Gly Lys Asn Pro Leu Leu Ser Lys Ser Ile Ile Ser Arg Glu Asn
100 105 110

Asn His Ser Ser Gln Gly Asp Ser Asn Lys Gln Ser Phe Ser Lys Lys
115 120 125

Val Ser Gln Val Thr Asn Val Ala Asn Arg Pro Met Leu Thr Asn Asn
130 135 140

Ser Arg Thr Ile Ser Val Ile Asn Lys Leu Pro Lys Thr Gly Asp Asp
145 150 155 160

Gln Asn Val Ile Phe Lys Leu Val Gly Phe Gly Leu Ile Leu Leu Thr
165 170 175

Ser Arg Cys Gly Leu Arg Arg Asn Glu Asn
180 185

<210> 15
<211> 298

<212> PRT
<213> Streptococcus agalactiae

<400> 15

Met Phe Asn Lys Ile Gly Phe Arg Thr Trp Lys Ser Gly Lys Leu Trp
1 5 10 15

Leu Tyr Met Gly Val Leu Gly Ser Thr Ile Ile Leu Gly Ser Ser Pro
20 25 30

Val Ser Ala Met Asp Ser Val Gly Asn Gln Ser Gln Gly Asn Val Leu
35 40 45

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
50 55 60

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
65 70 75 80

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Gly Asn Val Leu
85 90 95

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
100 105 110

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
115 120 125

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
130 135 140

Glu Arg Arg Gln Arg Asp Val Glu Asn Lys Ser Gln Gly Asn Val Leu
145 150 155 160

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Gly Asn Val Leu
165 170 175

Glu Arg Arg Gln Arg Asp Ala Glu Asn Arg Ser Gln Gly Asn Val Leu
180 185 190

Glu Arg Arg Gln His Asp Val Glu Asn Lys Ser Gln Val Gly Gln Leu
195 200 205

Ile Gly Lys Asn Pro Leu Phe Ser Lys Ser Thr Val Ser Arg Glu Asn
210 215 220

Asn His Ser Ser Gln Gly Asp Ser Asn Lys Gln Ser Phe Ser Lys Lys
225 230 235 240

Val Ser Gln Val Thr Asn Val Ala Asn Arg Pro Met Leu Thr Asn Asn
245 250 255

Ser Arg Thr Ile Ser Val Ile Asn Lys Leu Pro Lys Thr Gly Asp Asp
260 265 270

Gln Asn Val Ile Phe Lys Leu Val Gly Phe Gly Leu Ile Leu Leu Thr
275 280 285

Ser Leu Cys Gly Leu Arg Arg Asn Glu Asn
290 295

<210> 16
<211> 618
<212> PRT
<213> Streptococcus agalactiae

<400> 16

Met Phe Asn Lys Ile Gly Phe Arg Thr Trp Lys Ser Gly Lys Leu Trp
1 5 10 15

Leu Tyr Met Gly Val Leu Gly Ser Thr Ile Ile Leu Gly Ser Ser Pro
20 25 30

Val Ser Ala Met Asp Ser Val Gly Asn Gln Ser Gln Gly Asn Val Leu
35 40 45

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
50 55 60

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
65 70 75 80

Glu Arg Arg Gln Arg Asp Val Asp Asn Lys Ser Gln Gly Asn Val Leu
85 90 95

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
100 105 110

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
115 120 125

Glu Arg Arg Gln Arg Asp Val Asp Asn Lys Ser Gln Gly Asn Val Leu
130 135 140

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
145 150 155 160

Glu Arg Arg Gln Arg Asp Val Asp Asn Lys Ser Gln Gly Asn Val Leu
165 170 175

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
180 185 190

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
195 200 205

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
210 215 220

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
225 230 235 240

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
245 250 255

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
260 265 270

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
275 280 285

Glu Arg Arg Gln Arg Asp Val Asp Asn Lys Ser Gln Gly Asn Val Leu
290 295 300

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
305 310 315 320

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
325 330 335

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
340 345 350

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
355 360 365

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
370 375 380

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
385 390 395 400

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu

405 410 415

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
420 425 430

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
435 440 445

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
450 455 460

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
465 470 475 480

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
485 490 495

Glu Arg Arg Gln Arg Asp Ala Asp Asn Lys Ser Gln Gly Asn Val Leu
500 505 510

Glu Arg Arg Gln Arg Asp Ala Glu Asn Lys Ser Gln Val Gly Gln Leu
515 520 525

Ile Gly Lys Asn Pro Leu Phe Ser Lys Ser Thr Val Ser Arg Glu Asn
530 535 540

Asn His Ser Ser Gln Gly Asp Ser Asn Lys Gln Ser Phe Ser Lys Lys
545 550 555 560

Ile Ser Gln Val Thr Asn Val Ala Asn Gly Pro Met Leu Thr Asn Asn
565 570 575

Ser Arg Thr Ile Ser Val Ile Asn Lys Leu Pro Lys Thr Gly Asp Asp
580 585 590

Gln Asn Val Ile Phe Lys Leu Val Gly Phe Gly Leu Ile Leu Leu Thr
595 600 605

Ser Leu Cys Gly Leu Arg Arg Asn Glu Asn
610 615

<210> 17
<211> 901
<212> PRT
<213> Streptococcus agalactiae

<400> 17

Met Arg Lys Tyr Gln Lys Phe Ser Lys Ile Leu Thr Leu Ser Leu Phe

1 5 10 15

Cys Leu Ser Gln Ile Pro Leu Asn Thr Asn Val Leu Gly Glu Ser Thr
20 25 30

Val Pro Glu Asn Gly Ala Lys Gly Lys Leu Val Val Lys Lys Thr Asp
35 40 45

Asp Gln Asn Lys Pro Leu Ser Lys Ala Thr Phe Val Leu Lys Thr Thr
50 55 60

Ala His Pro Glu Ser Lys Ile Glu Lys Val Thr Ala Glu Leu Thr Gly
65 70 75 80

Glu Ala Thr Phe Asp Asn Leu Ile Pro Gly Asp Tyr Thr Leu Ser Glu
85 90 95

Glu Thr Ala Pro Glu Gly Tyr Lys Lys Thr Asn Gln Thr Trp Gln Val
100 105 110

Lys Val Glu Ser Asn Gly Lys Thr Thr Ile Gln Asn Ser Gly Asp Lys
115 120 125

Asn Ser Thr Ile Gly Gln Asn His Glu Glu Leu Asp Lys Gln Tyr Pro
130 135 140

Pro Thr Gly Ile Tyr Glu Asp Thr Lys Glu Ser Tyr Lys Leu Glu His
145 150 155 160

Val Lys Gly Ser Val Pro Asn Gly Lys Ser Glu Ala Lys Ala Val Asn
165 170 175

Pro Tyr Ser Ser Glu Gly Glu His Ile Arg Glu Ile Pro Glu Gly Thr
180 185 190

Leu Ser Lys Arg Ile Ser Glu Val Gly Asp Leu Ala His Asn Lys Tyr
195 200 205

Lys Ile Glu Leu Thr Val Ser Gly Lys Thr Ile Val Lys Pro Val Asp
210 215 220

Lys Gln Lys Pro Leu Asp Val Val Phe Val Leu Asp Asn Ser Asn Ser
225 230 235 240

Met Asn Asn Asp Gly Pro Asn Phe Gln Arg His Asn Lys Ala Lys Lys
245 250 255

Ala Ala Glu Ala Leu Gly Thr Ala Val Lys Asp Ile Leu Gly Ala Asn
260 265 270

Ser Asp Asn Arg Val Ala Leu Val Thr Tyr Gly Ser Asp Ile Phe Asp
275 280 285

Gly Arg Ser Val Asp Val Val Lys Gly Phe Lys Glu Asp Asp Lys Tyr
290 295 300

Tyr Gly Leu Gln Thr Lys Phe Thr Ile Gln Thr Glu Asn Tyr Ser His
305 310 315 320

Lys Gln Leu Thr Asn Asn Ala Glu Glu Ile Ile Lys Arg Ile Pro Thr
325 330 335

Glu Ala Pro Arg Ala Lys Trp Gly Ser Thr Thr Asn Gly Leu Thr Pro
340 345 350

Glu Gln Gln Lys Gln Tyr Tyr Leu Ser Lys Val Gly Glu Thr Phe Thr
355 360 365

Met Lys Ala Phe Met Glu Ala Asp Asp Ile Leu Ser Gln Val Asp Arg
370 375 380

Asn Ser Gln Lys Ile Ile Val His Ile Thr Asp Gly Val Pro Thr Arg
385 390 395 400

Ser Tyr Ala Ile Asn Asn Phe Lys Leu Gly Ala Ser Tyr Glu Ser Gln
405 410 415

Phe Glu Gln Met Lys Lys Asn Gly Tyr Leu Asn Lys Ser Asn Phe Leu
420 425 430

Leu Thr Asp Lys Pro Glu Asp Ile Lys Gly Asn Gly Glu Ser Tyr Phe
435 440 445

Leu Phe Pro Leu Asp Ser Tyr Gln Thr Gln Ile Ile Ser Gly Asn Leu
450 455 460

Gln Lys Leu His Tyr Leu Asp Leu Asn Leu Asn Tyr Pro Lys Gly Thr
465 470 475 480

Ile Tyr Arg Asn Gly Pro Val Arg Glu His Gly Thr Pro Thr Lys Leu
485 490 495

Tyr Ile Asn Ser Leu Lys Gln Lys Asn Tyr Asp Ile Phe Asn Phe Gly
500 505 510

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Ile Asp Ile Ser Ala Phe Arg Gln Val Tyr Asn Glu Asp Tyr Lys Lys
        515                 520                 525

Asn Gln Asp Gly Thr Phe Gln Lys Leu Lys Glu Glu Ala Phe Glu Leu
    530                 535                 540

Ser Asp Gly Glu Ile Thr Glu Leu Met Lys Ser Phe Ser Ser Lys Pro
545                 550                 555                 560

Glu Tyr Tyr Thr Pro Ile Val Thr Ser Ser Asp Ala Ser Asn Asn Glu
                565                 570                 575

Ile Leu Ser Lys Ile Gln Gln Gln Phe Glu Lys Val Leu Thr Lys Glu
            580                 585                 590

Asn Ser Ile Val Asn Gly Thr Ile Glu Asp Pro Met Gly Asp Lys Ile
        595                 600                 605

Asn Leu Gln Leu Gly Asn Gly Gln Thr Leu Gln Pro Ser Asp Tyr Thr
    610                 615                 620

Leu Gln Gly Asn Asp Gly Ser Ile Met Lys Asp Ser Ile Ala Thr Gly
625                 630                 635                 640

Gly Pro Asn Asn Asp Gly Gly Ile Leu Lys Gly Val Lys Leu Glu Tyr
                645                 650                 655

Ile Lys Asn Lys Leu Tyr Val Arg Gly Leu Asn Leu Gly Glu Gly Gln
            660                 665                 670

Lys Val Thr Leu Thr Tyr Asp Val Lys Leu Asp Asp Ser Phe Ile Ser
        675                 680                 685

Asn Lys Phe Tyr Asp Thr Asn Gly Arg Thr Thr Leu Asn Pro Lys Ser
    690                 695                 700

Glu Asp Pro Asn Thr Leu Arg Asp Phe Pro Ile Pro Lys Ile Arg Asp
705                 710                 715                 720

Val Arg Glu Tyr Pro Thr Ile Thr Ile Lys Asn Glu Lys Lys Leu Gly
                725                 730                 735

Glu Ile Glu Phe Thr Lys Val Asp Lys Asp Asn Asn Lys Leu Leu Leu
            740                 745                 750

Lys Gly Ala Thr Phe Glu Leu Gln Glu Phe Asn Glu Asp Tyr Lys Leu
        755                 760                 765
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Tyr Leu Pro Ile Lys Asn Asn Asn Ser Lys Val Val Thr Gly Glu Asn
770 775 780

Gly Lys Ile Ser Tyr Lys Asp Leu Lys Asp Gly Lys Tyr Gln Leu Ile
785 790 795 800

Glu Ala Val Ser Pro Lys Asp Tyr Gln Lys Ile Thr Asn Lys Pro Ile
805 810 815

Leu Thr Phe Glu Val Val Lys Gly Ser Ile Gln Asn Ile Ile Ala Val
820 825 830

Asn Lys Gln Ile Ser Glu Tyr His Glu Glu Gly Asp Lys His Leu Ile
835 840 845

Thr Asn Thr His Ile Pro Pro Lys Gly Ile Ile Pro Met Thr Gly Gly
850 855 860

Lys Gly Ile Leu Ser Phe Ile Leu Ile Gly Gly Ser Met Met Ser Ile
865 870 875 880

Ala Gly Gly Ile Tyr Ile Trp Lys Arg Tyr Lys Lys Ser Ser Asp Ile
885 890 895

Ser Arg Glu Lys Asp
900

<210> 18
<211> 674
<212> PRT
<213> Streptococcus agalactiae

<400> 18

Met Lys Lys Ile Asn Lys Cys Leu Thr Val Phe Ser Thr Leu Leu Leu
1 5 10 15

Ile Leu Thr Ser Leu Phe Ser Val Ala Pro Ala Phe Ala Asp Asp Val
20 25 30

Thr Thr Asp Thr Val Thr Leu His Lys Ile Val Met Pro Gln Ala Ala
35 40 45

Phe Asp Asn Phe Thr Glu Gly Thr Lys Gly Lys Asn Asp Ser Asp Tyr
50 55 60

Val Gly Lys Gln Ile Asn Asp Leu Lys Ser Tyr Phe Gly Ser Thr Asp
65 70 75 80

Ala Lys Glu Ile Lys Gly Ala Phe Phe Val Phe Lys Asn Glu Thr Gly
85 90 95

Thr Lys Phe Ile Thr Glu Asn Gly Lys Glu Val Asp Thr Leu Glu Ala
100 105 110

Lys Asp Ala Glu Gly Gly Ala Val Leu Ser Gly Leu Thr Lys Asp Thr
115 120 125

Gly Phe Ala Phe Asn Thr Ala Lys Leu Lys Gly Thr Tyr Gln Ile Val
130 135 140

Glu Leu Lys Glu Lys Ser Asn Tyr Asp Asn Asn Gly Ser Ile Leu Ala
145 150 155 160

Asp Ser Lys Ala Val Pro Val Lys Ile Thr Leu Pro Leu Val Asn Asn
165 170 175

Gln Gly Val Val Lys Asp Ala His Ile Tyr Pro Lys Asn Thr Glu Thr
180 185 190

Lys Pro Gln Val Asp Lys Asn Phe Ala Asp Lys Asp Leu Asp Tyr Thr
195 200 205

Asp Asn Arg Lys Asp Lys Gly Val Val Ser Ala Thr Val Gly Asp Lys
210 215 220

Lys Glu Tyr Ile Val Gly Thr Lys Ile Leu Lys Gly Ser Asp Tyr Lys
225 230 235 240

Lys Leu Val Trp Thr Asp Ser Met Thr Lys Gly Leu Thr Phe Asn Asn
245 250 255

Asn Val Lys Val Thr Leu Asp Gly Lys Asp Phe Pro Val Leu Asn Tyr
260 265 270

Lys Leu Val Thr Asp Asp Gln Gly Phe Arg Leu Ala Leu Asn Ala Thr
275 280 285

Gly Leu Ala Ala Val Ala Ala Ala Ala Lys Asp Lys Asp Val Glu Ile
290 295 300

Lys Ile Thr Tyr Ser Ala Thr Val Asn Gly Ser Thr Thr Val Glu Val
305 310 315 320

Pro Glu Thr Asn Asp Val Lys Leu Asp Tyr Gly Asn Asn Pro Thr Glu

325 330 335

Glu Ser Glu Pro Gln Glu Gly Thr Pro Ala Asn Gln Glu Ile Lys Val
340 345 350

Ile Lys Asp Trp Ala Val Asp Gly Thr Ile Thr Asp Val Asn Val Ala
355 360 365

Val Lys Ala Ile Phe Thr Leu Gln Glu Lys Gln Thr Asp Gly Thr Trp
370 375 380

Val Asn Val Ala Ser His Glu Ala Thr Lys Pro Ser Arg Phe Glu His
385 390 395 400

Thr Phe Thr Gly Leu Asp Asn Thr Lys Thr Tyr Arg Val Val Glu Arg
405 410 415

Val Ser Gly Tyr Thr Pro Glu Tyr Val Ser Phe Lys Asn Gly Val Val
420 425 430

Thr Ile Lys Asn Asn Lys Asn Ser Asn Asp Pro Thr Pro Ile Asn Pro
435 440 445

Ser Glu Pro Lys Val Val Thr Tyr Gly Arg Lys Phe Val Lys Thr Asn
450 455 460

Gln Ala Asn Thr Glu Arg Leu Ala Gly Ala Thr Phe Leu Val Lys Lys
465 470 475 480

Glu Gly Lys Tyr Leu Ala Arg Lys Ala Gly Ala Ala Thr Ala Glu Ala
485 490 495

Lys Ala Ala Val Lys Thr Ala Lys Leu Ala Leu Asp Glu Ala Val Lys
500 505 510

Ala Tyr Asn Asp Leu Thr Lys Glu Lys Gln Glu Gly Gln Glu Gly Lys
515 520 525

Thr Ala Leu Ala Thr Val Asp Gln Lys Gln Lys Ala Tyr Asn Asp Ala
530 535 540

Phe Val Lys Ala Asn Tyr Ser Tyr Glu Trp Val Ala Asp Lys Lys Ala
545 550 555 560

Asp Asn Val Val Lys Leu Ile Ser Asn Ala Gly Gly Gln Phe Glu Ile
565 570 575

Thr Gly Leu Asp Lys Gly Thr Tyr Ser Leu Glu Glu Thr Gln Ala Pro
580 585 590

Ala Gly Tyr Ala Thr Leu Ser Gly Asp Val Asn Phe Glu Val Thr Ala
595 600 605

Thr Ser Tyr Ser Lys Gly Ala Thr Thr Asp Ile Ala Tyr Asp Lys Gly
610 615 620

Ser Val Lys Lys Asp Ala Gln Gln Val Gln Asn Lys Lys Val Thr Ile
625 630 635 640

Pro Gln Thr Gly Gly Ile Gly Thr Ile Leu Phe Thr Ile Ile Gly Leu
645 650 655

Ser Ile Met Leu Gly Ala Val Val Val Met Lys Lys Arg Gln Ser Glu
660 665 670

Glu Ala

<210> 19
<211> 635
<212> PRT
<213> Streptococcus agalactiae

<400> 19

Met Lys Lys Gln Phe Leu Lys Ser Ala Ala Ile Leu Ser Leu Ala Val
1 5 10 15

Thr Ala Val Ser Thr Ser Gln Pro Val Ala Gly Ile Thr Lys Asp Tyr
20 25 30

Asn Asn Arg Asn Glu Lys Val Lys Lys Tyr Leu Gln Glu Asn Asn Phe
35 40 45

Gly His Lys Ile Ala Tyr Gly Trp Lys Asn Lys Val Glu Phe Asp Phe
50 55 60

Arg Tyr Leu Leu Asp Thr Ala Lys Tyr Leu Val Asn Lys Glu Glu Phe
65 70 75 80

Gln Asp Pro Leu Tyr Asn Asp Ala Arg Glu Glu Leu Ile Ser Phe Ile
85 90 95

Phe Pro Tyr Glu Lys Phe Leu Ile Asn Asn Arg Asp Ile Thr Lys Leu
100 105 110

Thr Val Asn Gln Tyr Glu Ala Ile Val Asn Arg Met Ser Val Ala Leu
115 120 125

Gln Lys Phe Ser Lys Asn Ile Phe Glu Lys Gln Lys Val Asn Lys Asp
130 135 140

Leu Ile Pro Ile Ala Phe Trp Ile Glu Lys Ser Tyr Arg Thr Val Gly
145 150 155 160

Thr Asn Glu Ile Ala Ala Ser Val Gly Ile Gln Gly Gly Phe Tyr Gln
165 170 175

Asn Phe His Asp Tyr Tyr Asn Tyr Ser Tyr Leu Leu Asn Ser Leu Trp
180 185 190

His Glu Gly Asn Val Lys Glu Val Val Lys Asp Tyr Glu Asn Thr Ile
195 200 205

Arg Gln Ile Leu Ser Lys Lys His Glu Ile Glu Lys Ile Leu Asn Gln
210 215 220

Ser Thr Ser Asp Ile Ser Ile Asp Asp Asp Asp Tyr Glu Lys Gly Asn
225 230 235 240

Lys Glu Leu Leu Arg Glu Lys Leu Asn Ile Ile Leu Asn Leu Ser Lys
245 250 255

Arg Asp Tyr Arg Val Thr Pro Tyr Tyr Glu Val Asn Lys Leu His Thr
260 265 270

Gly Leu Ile Leu Leu Glu Asp Val Pro Asn Leu Lys Ile Ala Lys Asp
275 280 285

Lys Leu Phe Ser Leu Glu Asn Ser Leu Lys Glu Tyr Lys Gly Glu Lys
290 295 300

Val Asn Tyr Glu Glu Leu Arg Phe Asn Thr Glu Pro Leu Thr Ser Tyr
305 310 315 320

Leu Glu Asn Lys Glu Lys Phe Leu Val Pro Asn Ile Pro Tyr Lys Asn
325 330 335

Lys Leu Ile Leu Arg Glu Glu Asp Lys Tyr Ser Phe Glu Asp Asp Glu
340 345 350

Glu Glu Phe Gly Asn Glu Leu Leu Ser Tyr Asn Lys Leu Lys Asn Glu
355 360 365

Val Leu Pro Val Asn Ile Thr Thr Ser Thr Ile Leu Lys Pro Phe Glu
370 375 380

Gln Lys Lys Ile Val Glu Asp Phe Asn Pro Tyr Ser Asn Leu Asp Asn
385 390 395 400

Leu Glu Ile Lys Lys Ile Arg Leu Asn Gly Ser Gln Lys Gln Lys Val
405 410 415

Glu Gln Glu Lys Thr Lys Ser Pro Thr Pro Gln Lys Glu Thr Val Lys
420 425 430

Glu Gln Thr Glu Gln Lys Val Ser Gly Asn Thr Gln Glu Val Glu Lys
435 440 445

Lys Ser Glu Thr Val Ala Thr Ser Gln Gln Ser Ser Val Ala Gln Thr
450 455 460

Ser Val Gln Gln Pro Ala Pro Val Gln Ser Val Val Gln Glu Ser Lys
465 470 475 480

Ala Ser Gln Glu Glu Ile Asn Ala Ala His Asp Ala Ile Ser Ala Tyr
485 490 495

Lys Ser Thr Val Asn Ile Ala Asn Thr Ala Gly Val Thr Thr Ala Glu
500 505 510

Met Thr Thr Leu Ile Asn Thr Gln Thr Ser Asn Leu Ser Asp Val Glu
515 520 525

Lys Ala Leu Gly Asn Asn Lys Val Asn Asn Gly Ala Val Asn Val Leu
530 535 540

Arg Glu Asp Thr Ala Arg Leu Glu Asn Met Ile Trp Asn Arg Ala Tyr
545 550 555 560

Gln Ala Ile Glu Glu Phe Asn Val Ala Arg Asn Thr Tyr Asn Asn Gln
565 570 575

Ile Lys Thr Glu Thr Val Pro Val Asp Asn Asp Ile Glu Ala Ile Leu
580 585 590

Ala Gly Ser Gln Ala Lys Ile Ser His Leu Asp Asn Arg Ile Gly Ala
595 600 605

Arg His Met Asp Gln Ala Phe Val Ala Ser Leu Leu Glu Val Thr Glu
610 615 620

Met Ser Lys Ser Ile Ser Ser Arg Ile Lys Glu
625 630 635

<210> 20
<211> 181
<212> PRT
<213> Streptococcus agalactiae

<400> 20

Met Lys Lys Ile Thr Thr Leu Ile Leu Ala Ser Ser Leu Leu Leu Val
1 5 10 15

Ala Thr Thr Ser Val Lys Ala Asp Asp Asn Phe Glu Met Pro Thr Arg
20 25 30

Tyr Val Lys Met Ser Glu Lys Ser Lys Ala Phe Tyr Gln Arg Leu Gln
35 40 45

Glu Lys Gln Arg Lys Ala His Thr Thr Val Lys Thr Phe Asn Asn Ser
50 55 60

Glu Ile Arg His Gln Leu Pro Leu Lys Gln Glu Lys Ala Arg Asn Asp
65 70 75 80

Ile Tyr Asn Leu Gly Ile Leu Ile Ser Gln Glu Ser Lys Gly Phe Ile
85 90 95

Gln Arg Ile Asp Asn Ala Tyr Ser Leu Glu Asn Val Ser Asp Ile Val
100 105 110

Asn Glu Ala Gln Ala Leu Tyr Lys Arg Asn Tyr Asp Leu Phe Glu Lys
115 120 125

Ile Lys Ser Thr Arg Asp Lys Val Gln Val Leu Leu Ala Ser His Gln
130 135 140

Asp Asn Thr Asp Leu Lys Asn Phe Tyr Ala Glu Leu Asp Asp Met Tyr
145 150 155 160

Glu His Val Tyr Leu Asn Glu Ser Arg Val Glu Ala Ile Asn Arg Asn
165 170 175

Ile Gln Lys Tyr Asn
180

<210> 21
<211> 48

<212> DNA
<213> Streptococcus agalactiae

<400> 21
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<210> 22
<211> 48
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<400> 22
ggcaatgttc tagagcgtcg tcaacgcgat gttgagaata agagccaa 48

<210> 23
<211> 48
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<213> Streptococcus agalactiae

<400> 23
ggcaatgttt tagagcgtcg tcaacgtgat gcggaaaaca agagccaa 48

<210> 24
<211> 48
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<213> Streptococcus agalactiae

<400> 24
ggcaatgttt tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 25
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<212> DNA
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ggcaatgttc tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 26
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<400> 26
ggcaatgttc tagagcgtcg tcaacgcgat gcagaaaaca gaagccaa 48

<210> 27
<211> 48
<212> DNA
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<400> 27
ggtaatgttc tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 28
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<212> DNA
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<400> 28
ggtaatgttc tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 29
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ggtaatgttc tagagcgtcg tcaacgcgat gttgagaata agagccaa 48

<210> 30
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<400> 30
ggcaatgttt tagagcgtcg tcaacgtgat gcggaaaaca agagccaa 48

<210> 31
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<400> 31
ggcaatgttt tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 32
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<213> Streptococcus agalactiae

<400> 32
ggcaatgttt tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 33
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<400> 33
ggcaatgttc tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 34
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<210> 35
<211> 48

<212> DNA
<213> Streptococcus agalactiae

<400> 35
ggcaatgttc tagagcgtcg tcaacgcgat gcagaaaaca gaagccaa 48

<210> 36
<211> 48
<212> DNA
<213> Streptococcus agalactiae

<400> 36
ggtaatgttc tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 37
<211> 48
<212> DNA
<213> Streptococcus agalactiae

<400> 37
ggcaatgttt tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 38
<211> 48
<212> DNA
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<400> 38
ggcaatgttt tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 39
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<212> DNA
<213> Streptococcus agalactiae

<400> 39
ggcaatgttt tagagcgtcg tcaacgtgat gcggaaaaca agagccaa 48

<210> 40
<211> 48
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<400> 40
ggcaatgttt tagagcgtcg tcaacgcgat gcagaaaaca gaagccaa 48

<210> 41
<211> 48
<212> DNA
<213> Streptococcus agalactiae

<400> 41
ggcaatgttt tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 42
<211> 48

<212> DNA
<213> Streptococcus agalactiae

<400> 42
ggcaatgttt tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 43
<211> 48
<212> DNA
<213> Streptococcus agalactiae

<400> 43
ggtaatgttc tagagcgtcg tcaacgcgat gttgaaaata aaagccaa 48

<210> 44
<211> 48
<212> DNA
<213> Streptococcus agalactiae

<400> 44
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<210> 45
<211> 48
<212> DNA
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<400> 45
ggtaatgttc tagagcgtcg tcaacgcgat gttgaaaata aaagccaa 48

<210> 46
<211> 48
<212> DNA
<213> Streptococcus agalactiae

<400> 46
ggcaatgttt tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 47
<211> 48
<212> DNA
<213> Streptococcus agalactiae

<400> 47
ggtaatgttc tagagcgtcg tcaacgtgat gcagaaaaca gaagccaa 48

<210> 48
<211> 48
<212> DNA
<213> Streptococcus agalactiae

<400> 48
ggcaatgttt tagagcgtcg tcaacgcgat gcagaaaaca gaagccaa 48

<210> 49
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<212> DNA
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